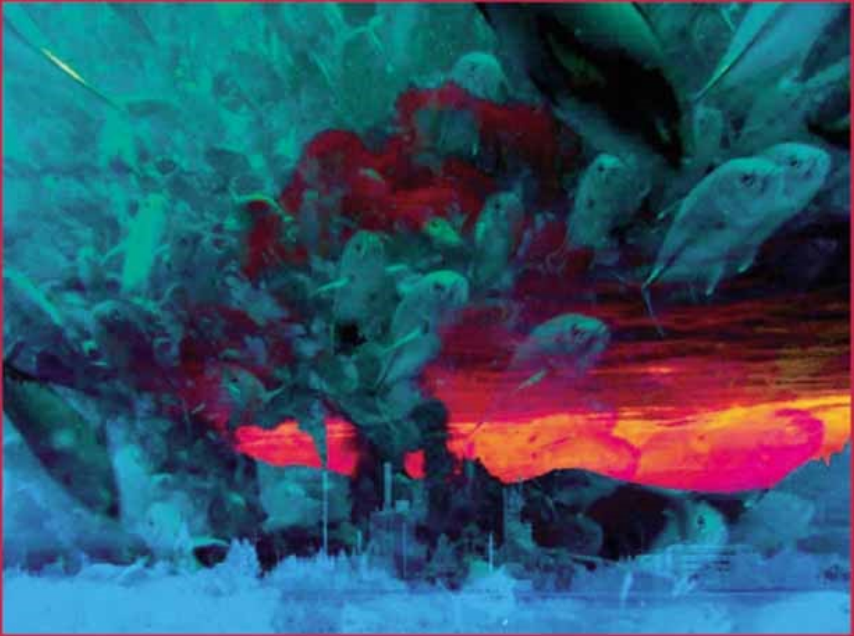
Environmental Pollution

Metals in Society and in the Environment

A Critical Review of Current Knowledge on Fluxes, Speciation, Bioavailability and Risk for Adverse Effects of Copper, Chromium, Nickel and Zinc

Lars Landner and Rudolf Reuther



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Metals in Society and in the Environment

ENVIRONMENTAL POLLUTION

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Metals in Society and in the Environment

A Critical Review of Current Knowledge on Fluxes, Speciation, Bioavailability and Risk for Adverse Effects of Copper, Chromium, Nickel and Zinc

by

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0 EXECUTIVE SUMMARY AND CONCLUSIONS

0.1 Introduction

Today, authorities responsible for regulating environmental quality in Europe, both at the Union and at the State level, are frequently referring to results from 'new research' in their efforts to regulate or restrict uses of metals and metal-containing materials. This has resulted in an increasing demand for critical, scientifically sound evaluations and compilations – in a transparent and holistic format – of relevant information about metal stocks and fluxes in society and the properties, behaviour and effects of trace metals in the environment.

One of the longest standing international programmes for assessing the effects of chemicals, including metals, on man and the environment, the "Environmental Health Criteria (EHC) Programme", was initiated in 1973. This effort is nowadays an integral part of the "International Programme on Chemical Safety" (IPCS), a cooperative programme of the three United Nations Agencies UNEP, ILO and WHO. While the first EHC monograph (on mercury), published in 1976, focused entirely on human health aspects, later EHCs for metals – at least since 1989 – also treated environmental aspects. For example, the EHCs for nickel (EHC # 108, publ. in 1991), for copper (EHC # 200, publ. in 1998) and for zinc (EHC # 221, publ. in 2001) included comprehensive sections on generic environmental exposure and environmental effects of the metals involved.

The environmental risk assessment of trace metals requires a cautious approach because of the natural occurrence of metals, the great variations in metal speciation, affecting the metal's bioavailability and toxicity and – for some metals such as chromium, copper, nickel and zinc – their essentiality for many organisms. These aspects were emphasized by the IPCS, e.g. in the EHCs for copper and zinc, as summarized by the following conclusions:

- ***The total concentration of an essential element such as copper or zinc, alone, is not a good predictor of its bioavailability or toxicity.***
- ***The toxicity of copper or zinc will depend on environmental conditions and habitat types, thus any risk assessment of the potential effects of these metals on organisms must take into account local environmental conditions.***

- ***Because copper and zinc are essential elements, procedures to prevent toxic levels in the environment should not incorporate safety factors that result in recommended concentrations being below natural levels or causing deficiency symptoms.***

Moreover, under the “Existing Substances Regulation” (793/93) of the European Union, a thorough assessment of the human health and environmental risks associated with production and use of **zinc** and **five zinc compounds** is being conducted by Dutch environmental authorities. In parallel with this “mandatory” environmental risk assessment (RA) for zinc, as an industry initiative, a voluntary RA of **copper** and **several copper compounds** is underway in cooperation with Italian authorities and the European Commission. These current trace metal environmental RA activities have called for updates of previous compilations of data on exposure and effects of metals in the environment. The new RAs have also directly encouraged important new research programmes, some of which are sponsored by the international metals industry.

Initiated by the Nordic metal industry, three monographs on “Metals in Society and in the Environment”, covering copper, zinc and major alloying metals in stainless steel (chromium, nickel and molybdenum), were prepared by the Swedish Environmental Research Group (MFG) in the late 1990s, and were published both in Swedish and in English (Landner and Lindeström, 1996; 1997; 1998; 1999; Walterson, 1999). The aim was to make a balanced and comprehensive compilation, together with a scientific appraisal, of available data on fluxes and stores of metals in the anthroposphere, on metal flows to and between environmental compartments, and on metal behaviour, exposure and effects in humans and in natural ecosystems. However, the current intense research on metal speciation and the upcoming new methodologies for prediction of metal bioavailability in water, sediments and soils, as a result of important scientific advances, made it urgent to bring this information up-to-date.

For this reason, the Swedish “Metal Information Task Force”[1] took an initiative, in the middle of 2002, to engage MFG to prepare an updated monograph on metals. In this follow-up work, special emphasis was given to new scientific advances made in the field of metal speciation in environmental media and to the interpretation of its consequences for the mobility, bioavailability and toxicity of trace metals in water, sediments and soils. Among the highlights that we will present and discuss in this monograph are two metal speciation-based concepts or tools recently developed to improve our understanding of environmental effects of metals,

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the “Acid Volatile Sulphides” (AVS) concept to predict metal bioavailability in sediments and the “Biotic Ligand Model” (BLM) to calculate the availability and toxicity of metals to aquatic biota. The metals included in this updating effort are, primarily, copper, nickel and zinc, and to some extent also chromium.

0.2 Metal fluxes from society to the environment and between environmental media

The use of ‘materials (or substance) flow analyses’ to establish metal mass balances in defined geographical areas has turned out to be an efficient approach to improving our understanding of resource availability and long-term environmental change. The construction of well-quantified metal cycles for a country – or a continent – may be helpful for designing sound policies for production, consumption and recycling of metals and can be used as a basis for decisions supporting an environmentally sustainable economic and social development.

A good example of such an exercise, where a team of researchers from Germany, Switzerland and the USA worked out a comprehensive copper cycle for an area covering the European Union and Poland, is presented and examined. The justification for this broad definition of the system boundaries is that both mining, primary and secondary production sites as well as a multitude of consumption sites are present within the boundaries. This exercise was made to incorporate all life stages and dissipative flows of copper in Europe, i.e. both recycling rates, various waste fluxes and dissipative flow rates have been estimated with greater accuracy than earlier. Consequently, the effort has been successful in providing a clearer picture of current copper fluxes and future needs to improve sustainability with regard to copper production and use on a continent-wide basis. Other recent attempts to develop practical tools for selecting the best options for nation-wide metals management, e.g. a research programme for dynamic mathematical modelling of metal flows in the Netherlands, turned out to be less successful.

In the effort to design sound management systems for metals on a continental scale, it may also be of great value to broaden our understanding of nature’s tolerance to long-term metal exposure. Hence, we have included a brief summary of a recent book by L. Lindeström, containing detailed descriptions of the variable environmental impacts caused by the mining and smelting activities that went on for one thousand years at the Falun Copper Mine.

On the other hand, studies of metal fluxes to and from systems covering a limited geographical area, e.g. a single city, may be used to focus on specific aspects, such as:

- the impacts of urban metal flows on society's metal recycling systems and on the local environment;
- the role and relative significance of diffuse sources of metal dispersion to the natural environment; and
- the transformation and speciation of metals during transport from sources in the anthroposphere to the final metal sinks.

A comprehensive analysis of the stores and fluxes of seven metals, including chromium, copper, nickel and zinc, within and out from the City of Stockholm was carried out, in 1995, as part of a broad Swedish research programme ("Metals in the Urban and Forest Environment"). This effort has generated invaluable information, that will be presented and critically discussed in Chapter 3 of this book.

The various attempts to establish metal balances for defined geographical areas have helped to identify gaps in the available knowledge and to initiate new research to fill in these gaps. In many cases, it was deemed necessary to quantify a specific metal flux more accurately, e.g. by using better experimental designs or improved measuring techniques. The new and more reliable data on metal fluxes that we will review in Chapter 4 relates to:

- fluxes resulting from corrosion of roofs and other metallic materials, followed by runoff from buildings and other urban constructions;
- fluxes from the traffic sector in built-up urban areas to biota in receiving waters; and
- metal releases from plumbing systems in houses followed by fluxes to sewage treatment plants, sewage sludge and agricultural soils.

0.2.1 The European copper cycle in the mid-1990s

Some of the major findings from the recently published copper cycle for Europe (EU + Poland), valid for the year 1994, may be summarized as follows:

- The European smelting industry was supplied with about 600 kt of copper from ores mined within the region and 280 kt in imported concentrates.
- However, most of the copper used in Europe, 1,900 kt, is mined, smelted and refined outside of the region.
- The industrial output of non-alloyed copper products amounts at 2,650 kt, while copper in alloys is about 780 kt (75% of copper in finished products is in pure form).
- Since about 920 kt of copper enters the waste management system, the yearly growth of the copper stock in Europe is about 2,500 kt or 6 kg per capita.
- The annual growth rate of copper in landfills and tailings ponds is 1.4 kg per capita.
- The fastest growing copper waste category is waste from electrical and electronic equipment (growth rate 5-10%), which requires more efficient recycling strategies.
- Since about 2 kg of copper is generated ’per capita’ annually in consumer waste in the region, and only about half of this amount, on average, is recycled, the study gives valuable information as to what waste categories should be given priority in improving the recycling rate, e.g. by developing appropriate technologies to separate waste streams.

0.2.2 Metal fluxes from mining waste – Falun Copper Mine

During the lifetime of the Falun Copper Mine, where mixed sulphidic ores were mined for more than a millennium, huge amounts of mining and smelting waste were emitted to the surrounding environment. Current estimates have arrived at total emissions in the order of 6,000 kt of sulphur dioxide and 15 kt of copper released to the atmosphere, and 500 – 1,000 kt of copper, lead, zinc and cadmium discharged to forest soils and watercourses, at the time causing dramatic impacts on the environment as well as on human health. However, a detailed assessment of the present environmental situation has revealed that the soils and terrestrial ecosystems in the vicinity of Falun have recovered to a remarkable degree during the 20th century after a series of emission-reducing measures had been introduced .

After the late 1980s, when treatment of the mine-water started, also the effluent-receiving river and lakes, that used to be exposed to very high metal concentrations in the water (for total copper 140-fold, and for total zinc 1,000-fold increases above regional background levels), exhibited clear signs of recovery. Only based on a clear appreciation of the speciation and the low bioavailability of harmful metals and of possible antagonistic (protective) interactions between metals such as zinc and other, more toxic metals, e.g. cadmium, lead and copper, was it possible to explain this rapid return to functioning aquatic ecosystems.

0.2.3 Urban metal flows – the case of Stockholm

According to the final reports from the project "Metals in the Urban and Forest Environment", the stocks and fluxes of metals in the City of Stockholm, in 1995, were as follows:

Stock/flux	Unit	Copper	Zinc	Chromium	Nickel
Total stock in city	ktonnes	123	28	5.6	2.5
Net growth rate	% per year	1.6	4.3	4.6	6.4
do.	kg per capita	2.8	1.7	0.4	0.2
Loss (as solid waste)	% per year	0.24	2.5	1.8	1.2
Loss (to environment)	% per year	0.01	0.09	0.02	0.04
do.	tonnes/year	12	24	0.8	0.6
Emissions to water	tonnes/year	2.6	9.1	0.4	1.5
Increase over backgrd.	%	15-24	41-54	29-40	14-15
Flux to aquatic sedim.	tonnes/year	1.7	4.0	1.0	0
To sewage sludge	tonnes/year	9.0	12	0.9	0.8

Per capita growth of the copper stock (2.8 kg/year), thus, was about half of that in Europe.

The types of goods causing the greatest releases of metals in Stockholm were found to be:

- for copper: water pipes in buildings, followed by motor vehicle brakes;
- for zinc: motor vehicle tyres, followed by various galvanized materials;
- for chromium and nickel: road pavements, followed by tyres.

A substantial part of the released metals is channelled through the city sewerage system (including storm-water sewers) to sewage treatment plants, where metals are partitioned between sludge and effluents to the recipient, where a certain fraction is deposited in the bottom sediments and the remainder transported further out to the Baltic Sea. Some recent attempts to determine the degree of bioavailability – in the aquatic environment – of the fraction of metals that is emitted from the street traffic have not been entirely conclusive.

0.2.4 Critical steps in metal fluxes from cities to the environment

New and accurate information on critical metal fluxes has been produced in studies, e.g. by Swedish researchers. The annual runoff of metals from copper, galvanized steel or stainless steel sheets (of different age), after atmospheric corrosion in a relatively clean urban air (in Stockholm, with sulphur dioxide, SO_2, levels of about 3 $\mu g/m^3$), has been measured during extended periods of exposure on roofs. While the corrosion rate shows a typical variation with time, the runoff rate remains relatively constant when atmospheric variables do not change. The runoff rate of copper from copper roofs is in the range 1.0-2.0 g Cu/m^2 and year, depending on the age of the roof. Runoff rates are clearly a function of the atmospheric SO_2 concentration, which has fallen dramatically in most major cities over the past 20-30 years, of rainwater pH and of the annual precipitation. Thus, in Singapore with up to 8 times higher air pollution and rainfall than in Stockholm, the copper runoff rate is about 5.7 g/m^2 and year.

For zinc, the annual runoff rate in Stockholm was determined to be 3.1 g Zn/m^2, while those of chromium and nickel from stainless steel were 0.2-0.7 mg/m^2 and 0.1-0.8 mg/m^2.

The speciation and fate of the released metals (copper, zinc) were studied in the runoff water on its transport from the roofs to the natural receiving water bodies. At the edge of a roof, copper and/or zinc in runoff largely occurs in the form of free, hydrated ions, but after the metal-laden water had percolated through soil or come in contact with concrete or limestone, 96-99.8 % of the total metal content in the runoff was retained and the remaining small residue of copper and zinc in the percolate had a very low bioavailability. These results indicate that environmental dispersal of bioavailable species of copper and zinc as a result of corrosion and runoff from roofs and galvanized structures can be effectively controlled by letting the runoff water percolate over concrete surfaces or through soil.

The extensive use of water pipes and heat exchangers made of copper may cause significant releases of copper, especially in areas with corrosive drinking water (rich in carbonate and/or organic ligands, forming soluble copper complexes). The released copper usually does not constitute any risk for human water consumers, but the copper in the sewage is finally incorporated into the sewage sludge. This is one reason why some concern has been expressed regarding the safety of sewage sludge in cases where such sludge is applied on arable land.

However, studies (over 18 growth seasons) of possible impacts of yearly application of copper-enriched sewage sludge to agricultural fields have come to the conclusion that an application rate in the range of 1.4 – 4.2 kg Cu per ha and year would not be harmful on soils used to raise cereals. The conclusion was drawn on the basis of no-observed-effects on soil fauna or soil microbes, on crop yields and copper content in crops even at the higher loading rate. Moreover, copper accumulation in the soil was marginal at the lower loading rate, with an insignificant risk of exceeding existing limit values for copper in agricultural soils to be fertilised with sludge, even in the long term. Thus, a provisional limit value for copper loading on most common types of agricultural soils would be 1.4 – 2.0 kg Cu per ha and year.

0.3 Speciation, Bioavailability and effects of trace metals in the environment

Assessments of the fate and biological effects of trace metals in the environment based solely on total concentrations of the metals in the various media (water, sediment or soil) is no longer state-of-the-art or scientifically justified. Although water, sediment and soil quality criteria for metals in most countries are still based on the total concentrations, it is now becoming more and more evident also for regulatory authorities that it is the actual metal species that determines mobility, bioavailability and toxicity of a metal, but also that metal speciation depends on the site-specific seasonal and spatial variations existing in a particular water, sediment or soil system.

The scientific community has vigorously responded to this new understanding by swiftly developing a whole array of new methods to describe and quantify the distribution and dynamics of various metal species occurring under different physico-chemical conditions in the environment. New, detailed models and descriptions of the mechanisms of formation of metal species and their transformations have been developed. A broad review of the new investigative tools and how to use them for widening our understanding of the behaviour and effects of trace metals in the environment is given in Chapters 5-7 of this monograph.

0.3.1 In the water column - BLM as a tool for prediction of toxicity

It has been repeatedly demonstrated that the toxicity, e.g. the acute median lethality, LC_{50}, of a trace metal to a single aquatic species, varies widely between different tests. Variations in toxicity of the same metal are directly related to variations in water hardness, pH, content of suspended solids and the concentration of organic ligands that can form complexes with the metal. All these water quality characteristics modify the speciation of a metal and, thereby, its bioavailability. The most available (and toxic) species of a divalent metal in aqueous solution usually is the free ion (e.g. Cu^{2+}, Ni^{2+}, Zn^{2+}), but also a few inorganic complexes (e.g. $CuOH^+$ and $CuCO_3$) have the potential to contribute to the total toxicity of these metals to aquatic organisms. It has also been shown that various common cations (Ca^{2+}, Mg^{2+}, Na^+ and H^+) compete with the trace metals for binding sites at the organism-water interface and, thus, tend to reduce the overall toxic response.

The recently developed “Biotic Ligand Models” (BLMs) for copper, nickel, silver and zinc interacting with fish, daphnids and algae, are able to handle all the above mentioned sources of apparent variation in toxicity and are now, after refinement, powerful tools for making accurate predictions of the toxicity of a trace metal in natural waters with widely differing chemical composition. A basic assumption of the BLM is that metal toxicity occurs as a result of metal ions reacting with binding sites at the organism-water interface, e.g. the gills, thus forming a metal-biotic ligand (metal-BL) complex. The concentration of this metal-BL complex directly reflects the magnitude of the resulting toxic effect, independent of the physico-chemical characteristics of the surrounding water. Hence, the metal toxicity can be predicted when metal speciation, the activity of each cation in solution, and the stability constant for each cation binding to the BL of the actual organism are known.

It has been demonstrated that the concentrations of trace metals in gills of fish were constant predictors of the acute toxicity of the metals to the fish, although water hardness varied up to tenfold. In contrast, total metal concentrations (e.g., for copper, nickel or zinc) in the water or the free-ion activities of these metals could not be used as accurate toxicity predictors. Also with regard to the prediction of chronic toxicity, the BLM has been successfully used. Chronic toxicities of copper and zinc to rainbow trout, *Daphnia magna* and green microalgae could be predicted within a factor of two compared to the observed values, when performing toxicity tests both in laboratory waters and in waters collected from the field. Dietary uptake of copper in daphnids did not enhance the chronic toxicity of waterborne copper, an observation that does not exclude, however, that dietary uptake of trace metals may influence the toxic response, e.g., in typically particle-ingesting organisms.

0.3.2 In aquatic sediments – AVS as a tool for prediction

Bioavailability and toxicity of sediment-associated trace metals towards bottom-dwelling organisms are governed by site-specific factors like sediment properties, the redox potential, the chemistry of pore-water and overlying water, and by the physiology and feeding behaviour of the fauna. In oxidized sediment layers, iron and manganese oxy-hydroxides and particulate organic carbon mainly control the bioavailability of trace metals, while in anoxic sediments, the most important regulating factors are pH and sulphides. Undisturbed sediments rich in organic matter may have a very sharp gradient in redox potential, so that anoxic conditions appear at a depth of 2-5 mm, although the sediment surface is well oxidized.

A multitude of speciation methods is now available to determine typical species of trace metals in sediments, their relative distribution among various sediment fractions, or the kind and kinetics of transformations between different metal forms. Examples are wet chemical methods like sequential extractions, which successively extract metals from the sediment matrix by using increasingly powerful extractants. The outcome provides information about the strength of binding between a metal and the major sediment fractions, and hence on its potential mobility and bioavailability. Other, more sophisticated approaches include measurements of the sediment's capacity to buffer protons and electrons, thermodynamic and kinetic solubility calculations, new spectroscopic techniques (like X-ray absorption spectroscopy) and the determination of the acid-volatile sulphides (AVS).

The AVS approach has attracted a lot of interest, since it turned out to be a rapid, simple and cheap method for predicting at least the <u>absence</u> of trace metal-related toxicity of aquatic sediments. It is well known that sulphide, derived from sulphate-reducing bacteria in the pore-water of anoxic sediment layers, forms relatively insoluble complexes with various trace metals, thereby rendering them non-bioavailable and non-toxic. These metal sulphides (AVS) can be liberated from sediments by treatment with 1 N HCl. In fact, sediments containing an excess of AVS over "simultaneously extracted metals" (SEM) are characterized by very low pore-water metal concentrations, as well as low metal bioavailability and toxicity. Both the U.S.EPA and the Environmental Directorate of the European Commission have now proposed the $\sum$SEM / AVS ratio, or the $\sum$SEM – AVS difference, as a reliable measure of the bioavailability of sediment-associated metals, e.g. cadmium, lead, copper, nickel and zinc.

In general, the model has been found to be a good predictor of the non-toxicity of trace metals in sediments, especially after normalisation to the sediment organic matter content, but may not be fully reliable in predicting that a certain sediment is toxic to the benthic fauna. In other words, the SEM/AVS approach tends to overestimate the toxicity of a sediment, i.e. a greater number of sediments are predicted to be toxic than what is really the case.

Although the SEM/AVS approach has been quite successful, so far, in predicting the bioavailability and toxicity of trace metals in sediments, recent studies have pointed to some weaknesses in the model that leave room for future improvements. Moreover, there is a need to apply more fine-scale sediment sampling techniques, such as the "diffusive gradients in thin films" (DGT) technique, which allows the documentation of metal releases from solid phases in discrete locations within a sediment core, thus providing a better understanding of the exact interactions between burrowing organisms and sediment-associated trace metals.

0.3.3 In soils – laboratory versus field tests

In spite of the many differences between sediment and soil systems, their geochemical similarity has justified the development and use of similar methods for metal speciation in both media. A first approximation for the speciation of soil-associated metals may be achieved by experimentally determining solid-liquid partitioning coefficients (k_d). Such coefficients help to differentiate between metals dissolved in the soil solution and those bound to particulate phases. But they tell us very little about the dynamic relationships existing between free, labile and non-labile metal species. Measured k_d values depend above all on the dissolved organic matter (DOM) content and on the total dissolved metal concentration in the soil solution. Thus, it is evident that every factor influencing soil DOM will also affect metal partitioning. Therefore, competitive adsorption models rather than simple solid-solution ratios (as expressed by k_d) are increasingly used to predict concentrations of dissolved metals.

Dissolved metal concentrations in soils vary widely with seasonal changes in redox potential, which may be triggered by light and temperature variations and, hence, by the activity of micro-organisms. Effects of soil aging on trace metal fluxes usually become manifest as a stronger adsorption and reduced extractability of metals. Depletion of trace metals in the vicinity of plant roots, due to uptake, promotes metal transfer from solid phases into solution. Plants can actively mobilise essential metals such as iron, copper and zinc from solid phases under deficiency conditions. Due to these site-specific phenomena, it is increasingly important to describe the kinetic, rather than only the thermodynamic equilibria for metals in soils.

In order to predict trace metal bioavailability to plants, the new “effective concentration” (C_E) concept has attracted considerable interest, because it fulfils the requirement of including kinetic aspects into the assessment. It relies upon using the “diffusive gradients in thin films” (DGT) technique, by which a metal chelating resin is introduced into the soil to mimic metal uptake by plants. Like plants, the DGT responds to the labile metal pool, re-supplied from both the soil solution and the solid phases. Measured DGT fluxes can be quantitatively related to C_E, and thus, provide a measure of the potential hazard of metals in contaminated soils.

In general, soil quality guidelines for trace metals are still not expressed in terms of bioavailable metals. A problem is, of course, that the bioavailable fraction of a trace metal in soil is typically variable and organism-specific, i.e. it may not be the same for a higher plant, a soil invertebrate or micro-organism species. Some soil scientists have stressed that it is necessary to consider the regional, natural background concentration of the metal in question and to formulate permissible levels in terms of “critical enhancement”, i.e. the number of times that a metal is allowed to increase in the soil relative to the background level. However, metals freshly introduced into the soil matrix have a different behaviour (mobility, bioavailability and toxicity) than metals gradually introduced over a long period of time, so the question on metal aging in the soil has to be taken into account. This was demonstrated in some recent studies of copper, lead and zinc. In one major study, zinc toxicity was shown to be consistently lower in field contaminated soils than in corresponding zinc-spiked soils. The importance of soil properties for the degree of toxicity, that a specific zinc addition will cause, became evident. We may even deepen our understanding of the observed metal-related toxicity variations in soils by normalizing the toxicity data to the particular soil type, thereby deriving specific “soil sensitivity factors”.

0.4 General conclusions

One of the most popular subjects of investigation among environmental chemists and eco-toxicologists for at least three decades has been the behaviour and effects of trace metals in the environment. The upsurge of this research happened concomitantly with the rapid and widespread introduction and use of atomic absorption spectrometric (AAS) methods. Over several decades, a huge amount of analytical data have accumulated describing total metal concentrations, e.g. of chromium, copper, nickel or zinc in water, in sediment or in soil samples (after extracting the target metal(s) in the sample). Now, most of the data has turned out to be of rather limited or no value for the understanding of trace metal interactions with biota. So, real advances in metal eco-toxicology were rather poor during that period.

It may be stated that the science of metal eco-toxicology did not really take off the ground until eco-toxicologists understood that trace metals, including the essential elements, occur in many different forms (species) in the environment and that only a few of these species, under certain circumstances, are bioavailable, i.e. can be taken up by a target plant, animal or micro-organism. Thus, it was not until people started to take a more sceptical attitude to the previously felt all-pervading blessings of the AAS and new analytical techniques were developed to consider metal speciation in environmental media that metal eco-toxicology entered its ‘Golden Age’.

Today, when performing generic environmental risk assessments of trace metals (such as chromium, copper, nickel or zinc) or when evaluating the environmental impact of trace metal emissions in a defined geographical area, it is no longer acceptable to routinely start out from laboratory toxicity data and divide them by conventional safety (or application) factors to derive “predicted no-effect concentration”. First, the natural background concentration of the relevant species of the assessed metal is usually fully considered. In case it is an essential element, it is state-of-the-art to consider the possibility of rectifying a situation of deficiency. An indispensable part of the assessment is to investigate (or predict) the potential distribution of bioavailable metal species under site-specific, realistic environmental conditions and to base this assessment – as far as possible – on long-term data from the field (rather than on laboratory data or short-term metal-spiking experiments), in order to take into account kinetic equilibria that may form in the real world and under realistic time scales. Since it is a highly dynamic habitat in which trace metals have to be assessed, direct measurements of the occurring metal species may only give a snapshot, and therefore, an erroneous picture of the true situation. Consequently, it may be more correct to base the assessment on a thorough knowledge of stability constants and ion activities of critical metal species, and on the use of mathematical models, such as the BLM, to calculate the potential risk of adverse effects, in view of possible, relevant combinations of site-specific environmental characteristics.

1 PURPOSE OF THIS REVIEW

The past five to eight years can be characterised as a period of very active research on metal use in society as well as on metal fluxes and effects in the environment and on human health. Thus, a considerable amount of valuable new data covering various environmental aspects of trace metals has been published since the three monographs, summarising the then available knowledge on zinc (Zn), copper (Cu) and chromium (Cr) – nickel (Ni) – molybdenum (Mo) "in Society and in the Environment", were prepared by MFG, Sweden, in the years 1996 – 1999.

Regulatory authorities in Europe, both at the union and the national level, are frequently referring to results from "new research" in their efforts to regulate or restrict the uses of metals and metal-containing materials. As a support to rational decision-making, it seems essential to ensure that new data is properly reviewed and evaluated regarding its scientific quality, and that the new information is compiled in a readily accessible form. Therefore, the Swedish "Metal Information Task Force" (MITF) took an initiative, in the middle of 2002, to let MFG make an update of the information given in the previous monographs, with the aim of presenting a comprehensive overview of the latest advances in the research on the behaviour and effects of trace metals in the environment.

The main focus of current metal research has been on (i) metal fluxes within society and from the anthroposphere to the environment, and on (ii) metal speciation in natural waters, sediments and soils, including metal binding to biomolecules (in gill tissue, intestines, etc.) and metal uptake into living organisms. These aspects have also been given particular emphasis in the present update.

1.1 Background and justification

During the second half of the 1990s, three separate monographs on copper, zinc and major alloying metals in stainless steel (chromium, nickel and molybdenum) in society and in the environment were compiled (by MFG) and published in Sweden. The monograph on zinc came in its first edition, in Swedish, in 1996 and was based on information (scientific papers and other reports) produced up to and including 1995. A translation into English was published in 1998 (Landner and Lindeström, "Zinc in Society and in the Environment" (1998); - in short: L & L – Zn). The book on copper in its first edition, in Swedish, covered research and other information up to 1997. The second edition of the copper book, published in 1999 (Landner and Lindeström, "Copper in Society and in the Environment" (1999); - in short: L & L – Cu), was partly revised and included some new information also from 1998. The third monograph (Walterson, "Chromium, Nickel and Molybdenum in Society and in the Environment" (1999); - in short: EW – Cr,Ni,Mo), presenting information on the three major alloy metals in stainless steel, also covered the time period up to the beginning of 1998. These three previous monographs are referred to in the following text by using the above indicated short forms.

During the five to seven years after these books were published, very intensive research has been conducted in many countries (notably Canada, the USA and Europe, incl. Scandinavia) on various aspects of metal behaviour and effects in the environment. Much of this research has been focused on the question of speciation in water, sediments and soil of trace metals, occurring as divalent cations under normal environmental conditions, such as cadmium, copper, lead, nickel and zinc. At the same time, representatives of political parties and mass media, of local, regional, national and international administrations, as well as members of NGOs, private companies and associations have – to an increasing extent – directed their interest to questions related to impacts of metals on the environment and on human health.

This increasing demand, by various debaters and decision-makers, for information and knowledge on the properties and behaviour of metals in society and in the environment has prompted, first, the realisation of a number of applied research projects designed to examine several new aspects of metal biogeochemistry and to provide answers to specific questions asked by these actors. Among such questions were the request for more detailed accounts of fluxes of trace metals from society to the environment as well as improved measurements of fluxes and pools of metals in various environmental compartments. Secondly, this research driven by the needs of political and administrative actors has produced results that have sometimes been given contradictory interpretations and, therefore, there is a growing need for critical evaluation of the new results, based on scientifically sound criteria, and for a compilation of the relevant information in a transparent and holistic form.

Prominent examples of fields of research on metals in the aquatic environment that have attracted considerable interest over the past five to ten years are (i) development of methods for prediction and assessment of trace metal toxicity in aquatic sediments; (ii) definition of sediment quality guidelines for trace metals in oxidised as well as anoxic fresh-water and marine sediments; (iii) assessment of degree of metal contamination (enhancement of trace metal concentrations, relative to natural background concentrations) in matrices such as sediments and soils; (iv) determination of the bioavailable fraction of metal, dissolved in the water, and development of "Biotic Ligand Models"(BLMs); (v) description of how trace metals in the aquatic environment are distributed between suspended particles, colloidal material and aqueous solution and of the factors governing their redistribution between phases; (vi) determination of the role of biotic components in the interaction with environmental trace metals (adaptation, acquired tolerance, antagonistic effects).

Research on the behaviour and effects of trace metals in soils has also resulted in a considerable number of scientific papers over the past five to ten years. Some of this information was presented in the International Copper Association (ICA) volume on "Copper in Sewage Sludge and Soil", published in 2000 (Landner et al., 2000), but several additional long-term field studies of the uptake and effects of trace metals in crops have been reported more recently.

1.2 The need and how to meet it

Today, authorities in different European countries, including Sweden, are frequently referring to results from "new research" in their efforts to regulate or restrict various uses of metals and metal-containing materials. If a balanced and rational decision process should be possible, it is of great importance that the information emanating from new research is readily available for all involved parties, and that this new information has been properly reviewed and evaluated with respect to its scientific quality.

The various scientific papers and other research reports on fluxes, behaviour and effects of trace metals that were produced over the past five to seven years have been published in many different journals, books or report series. Therefore, it is quite difficult to get a good overview over the entire data base and all the relevant aspects covered by the new data. Although a certain number of initiatives are currently taken internationally to compile information on separate trace metals (such as the EU Risk Assessment of zinc and the WHO Environmental Health Criteria documents on copper and zinc) or on specific topics such as the BLM, information is still scattered and difficult to access for those who want a comprehensive overview.

This was one of the major reasons for the Swedish "Metal Information Task Force" (MITF) to take an initiative, in the middle of 2002, of making an update of the previous monographs on metals.

1.3 Target groups for the updated report

The main target group for the document would be scientists and other professionals in the field of environmental and health sciences, working as informers or advisors to decision-makers at the local, regional, national or international levels both within the public and the private sector. In other words, the information compiled and critically reviewed in the report is intended to assist people used to read scientific texts, on one hand, to quickly identify the controversial parts of the new information and, on the other, to understand what parts of the new information (if any) that have reached the level of "conclusive evidence" in the sense that a consensus-based picture is emerging. In addition to its use by the immediate target group, i.e. professionals in Scandinavia, it is assumed that the report also could serve both an educative purpose in several other European countries or regions, where there is an urgent need to get a comprehensive overview of the state-of-the-art of current research on some of the central fields of interest regarding metals in the environment and contribute to improved risk assessments of metals.

1.4 Implementation of the work

As outlined above, the main focus of the updating procedure was decided to be on three major fields of research that have attracted considerable interest for the past 5-7 years:

- Flows of trace metals in society, with particular emphasis on the fluxes from the anthroposphere to the environment and on the factors determining the magnitudes of these fluxes.
- Metal speciation in natural waters, sediments and soils, including metal binding to naturally occurring ligands, including biomolecules (in gill tissue, intestines, etc.).
- Use of information on metal speciation for a better understanding of the mobility, bioavailability and biological effects of trace metals.

The metals involved are primarily chromium, copper, nickel and zinc, but information on molybdenum (where existing) will also be included. A brief discussion on essentiality (deficiency – optimal level – regulation – adaptation - toxicity) will be included, as will a discussion on how to assess anthropogenic enhancement of environmental concentrations of metals, relative to natural background concentrations.

The literature sources providing the background material for the update will mostly consist of original papers in peer-reviewed scientific journals of international reputation, but also reports from the "grey literature" (to be used only after careful quality assurance). Moreover, high-quality information compiled in various reports published by or in co-operation with the International Council on Mining and Metals (ICMM) and/or other industrial associations will also be used (again after careful scrutiny). A very important source of new information on bioavailability of metals in the environment has been made available through the significant advances made as a result of current research conducted to meet the information needs of the EU Risk Assessment for zinc and other metals (see e. g. Eurométaux, 2003; IZA, 2003).

Additional information will be gathered from recent Swedish reports, such as PhD and Lic. theses on corrosion and runoff from copper and zinc covered roofs and facades, presented at the Div. of Corrosion Sciences, Royal Institute of Technology, Stockholm, or long-term agricultural studies on metal mobility and effects on sludge-amended soils, published by regional agricultural associations. Moreover, a recent account of the environmental consequences of one thousand years of mining activities at Falu Gruva in central Sweden, prepared by Lindeström (2003), will serve as an important source of information on long-term metal contamination of a large river catchment area. Part of the information on bioavailability and toxicity of copper and zinc in aquatic sediments will be gathered from ongoing compilation and evaluation work, conducted by Landner and commissioned by SCDA and Zinc-Info Norden. Most of the compilation and critical review of information from various sources to be used in the present report on metals in society and in the environment was conducted during the second half of 2002 and the first half of 2003.

2 GLOBAL EXTRACTION, PRODUCTION AND CONSUMPTION

As a background to the discussion on metal cycles in the anthroposhpere of defined geographical areas (chapter 3) and on metal fluxes from society to the environment (chapter 4), it was felt pertinent to give a brief overview of the latest developments in the global metal mining activities as well as the metal consumption patterns. The principle feature in the production of base metals since the mid-1990s is a less dynamic development than earlier, and the overall demand – particularly for Zn and Cu – has stagnated. The lack of correspondence between production and demand for most base metals, illustrated by the build-up of considerable stocks, has resulted in stagnating or falling metal prices at least up till the end of 2002, maybe with the exception for Ni.

The levels and trends in primary and secondary production, as well as in consumption, of copper, nickel and zinc up to 1995-96 were described in the previous monographs (L & L – Zn, L & L – Cu and EW – Cr,Ni,Mo). After the middle of the 1990s, the production volume of the three metals has developed in a less dynamic way than before, and the supply of metals has not closely followed the demand on the world market. While copper production has continued to rise over the last 3-4 years at a rate of almost 4% yearly, the growth of nickel has lately shown a certain stagnation. Zinc production has grown much more than the current demand, which in fact decreased by 1% in 2001, before returning to growth in 2002. This lack of balance between supply and demand has resulted in the build-up of considerable stocks and in declining prices.

2.1 Copper

The global production of copper from ores has increased by 64% over the past two decades, from about 8.3 million tonnes (Mt) in 1982 to a projected production of 13.6 Mt in 2001. At the end of 2001 and in early 2002, the copper stocks were unusually high (about 1 Mt), and this phenomenon has tended to weaken mine production. The dominant position of Chile in the world's copper production has been further strengthened during the past five years (altogether, Chile has trebled its share of the global copper production since 1982: from 12.6% to 35%), and the output in 2001 was more than 4.7 Mt (SGU, 2002).

The International Copper Study Group (ICSG) made an estimate of the development of the global refining capacity over the period 2002 – 2006. They arrived at a likely increase by some 2 Mt, from 18.23 Mt in 2002 to 20.25 Mt in 2006 (SGU, 2003).

European countries have kept a relatively constant level of copper production during the past 5 years. If Russia is not included among the European countries, it is Poland that is by far the leading copper producer in Europe, with a production about 2.5 times that of the present EU Member States. Among the EU Members, Portugal and Sweden are producing at about the same level, 80 kt/year. The central and southern African countries have largely lost their former strong position as copper producers. This is particularly true for the Democratic Republic of Kongo (former Zaire), where current copper production is only about 6% of what it used to be in the early 1980s (SGU, 2002).

Among major copper consumers, P.R. of China has taken the leading role with a rise in copper consumption over the period 1998 to 2002 of about 17% per year. Asia, except Japan, accounts today for some 40% of the global copper consumption (SGU, 2003).

2.2 Nickel

The trade conditions for nickel reflect very closely those of stainless steel, since the dominant use of nickel is for this application. Use of stainless steel has shown a steady growth over many years, recently, but in 2001, the trend was reversed and showed a 3% decline. The demand of stainless steel has increased sharply in China over the past few years, and the nickel price at the London Metal Exchange has also increased rapidly since the historically low level (below 5,000 USD/tonne) at the end of 2001 to above 9,000 USD/tonne in early 2003 (SGU, 2003). The production capacity of both nickel ore and nickel metal is rapidly being reorganised, partly due to the very important changes taking place in the traditionally very big producer, Norilsk Nickel, both within and outside Russia.

The global production of nickel from ores has grown by almost 14% between 1999 and 2001 (to a level of 1.2 Mt), which is much more than the simultaneous growth in consumption, which was less than 4% (to 1.12 Mt in 2001) (SGU, 2002). However, in 2002 and early 2003, production of refined nickel rose, so that the global production estimate for the whole of 2003 was set at 1.24 Mt, while the consumption of the same grade was projected at

1.26 Mt (SGU, 2003). Figure 2.1 gives an overview of recent temporal trends in global production and use of refined nickel.

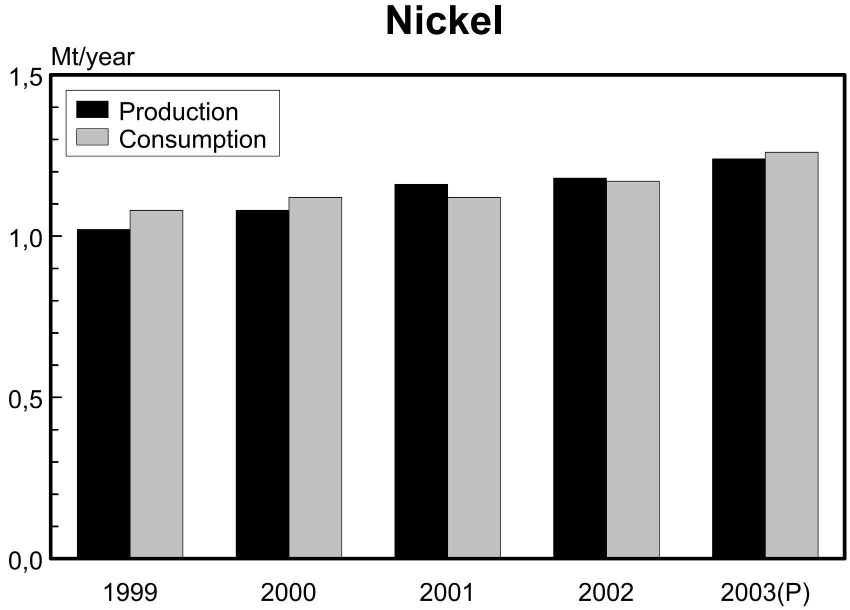


Figure 2.1. Development of global production and consumption of refined nickel (in Mt/year) in the period 1999-2003. Figures for 2003 projected. After SGU, 2002; 2003.

In Sweden, the use of primary nickel grew by about 6% during the year 2002 to almost 38 kt, while the consumption of stainless scrap increased by some 3% to over 580 kt. The total production of stainless steel in Sweden in the year 2002 amounted at almost 820 kt (SGU, 2003).

2.3 Zinc

Zinc production from ores grew by 9.2% from 1999 to 2002 and reached a volume of 8.81 Mt, and appears to exhibit an accelerated growth in the early 2003. Production of refined zinc attained 9.24 Mt in 2001 and 9.60 Mt in 2002. However, the demand for refined zinc stagnated occasionally in 2001, at a level of just below 9 Mt, but after that, growth in consumption of refined zinc came back to the previous high rate (a growth rate of close to 6% between the first two months of 2002 and the same months of 2003) (SGU, 2003). The temporal trends in production and use of refined zinc in recent years are depicted in Figure 2.2.

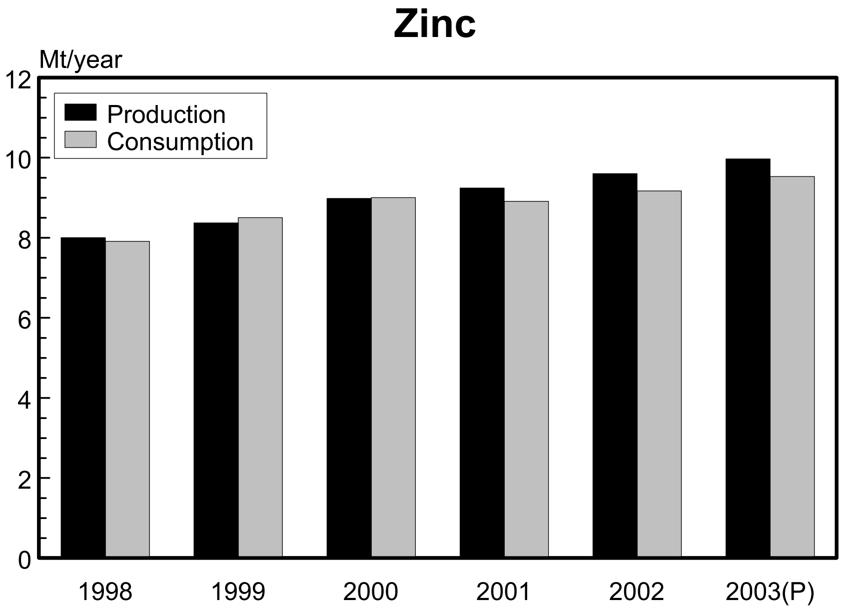


Figure 2.2. Development of global production and consumption of refined zinc (in Mt/year) in the period 1998-2003. Figures for 2003 projected. After SGU, 2002; 2003.

The three major zinc mining countries in the world are China, Australia and Peru, together covering almost 50% of the world production. Production of zinc from ores in Europe amounts only to slightly more than 1 Mt/year, but the European production of refined zinc is almost 3 times larger. Some of the biggest zinc refineries in the world are located in Europe (in Belgium, Finland, Norway and Spain). Among the major metals being examined in this report, zinc is the one that is used in highest volumes in Europe.

3 METAL CYCLES IN DEFINED GEOGRAPHICAL AREAS: EUROPE, THE NETHERLANDS AND STOCKHOLM

3.1 Example 1: The European copper cycle

Industrial ecologists have, to an increasing extent, been using materials (and substance) flow analyses to construct cycles of metals in defined geographical areas in order to get a better understanding of resource availability and long-term environmental change. Construction of well-quantified metal cycles would be very helpful in the design of sound policies related to production, consumption and recycling of metals and as a basis for decisions on a sustainable environmental policy. Examples of such recent attempts to construct metal cycles are those for Cu, Zn, Cr and Ni in Sweden, published in the previous monographs on metals and those made for greater Stockholm in the project "Metals in the Urban and Forest Environment".

A comprehensive Cu cycle for Europe (the EU together with Poland) was recently worked out by a team of researchers from Yale University, Cottbus and Zürich. Their four reports, published in 2002 in "Ecological Economics", are reviewed and discussed below in some detail. In this work, efforts were made to incorporate all life stages and dissipative flows of Cu, meaning that both the recycling rates, the various waste flows and the dissipative flow rates have been estimated with greater accuracy than earlier and the new Cu cycle now covers a more relevant geographical region, i.e. Europe.

Among the many conclusions of this work, the following ones deserve to be emphasized:

- *Most of the Cu used in Europe is mined, smelted and refined outside of the region, meaning that a net total of 1,900 kt of Cu was imported in 1994.*
- *To supply the European Cu smelting industry, some 600 kt of Cu originated from ore mined within the region and 280 kt was contained in imported concentrates.*

- *The output of non-alloyed Cu products is 2,650 kt/y, while the finished Cu alloy products amount at 780 kt/y (75% of Cu in finished products is in pure form).*
- *Since about 920 kt/y of Cu enters the waste management system, the yearly increase of the stock of Cu in European society is about 2,500 kt (6 kg/capita, y).*
- *Annual growth of waste Cu in landfills and tailings ponds is about 580 kt, which corresponds to a growth rate of 1.4 kg/capita.*
- *Waste from electrical and electronic equipment is the fastest growing waste category (annual growth rate 5-10%); thus, here is a need for efficient recycling strategies.*
- *The overall recycling efficiency for Cu in Europe is 48%, with relatively great variation between countries; thus, there is further potential for increased recycling efforts in the future.*
- *Improved recycling of certain waste categories by applying appropriate technology is a much easier and quicker resource-saving measure than redesign of industrial and commercial products.*

3.1.1 Introduction

Materials flow analysis (MFA) and – as a component of this – substance flow analysis (SFA) are approaches increasingly used in industrial ecology during the last decade. After having defined the system to be analysed (e.g. the technosphere or human society within a continent, a country or a city), the material or substance is chosen and then the analysis attempts to give answers to some or all of the following questions (cf. Graedel, 2002):

- How much material enters the system?
- How is the material transformed?
- How much material escapes from the system to the environment?
- How much material is added to the stock in use?
- How much material is archived in landfills and other reservoirs?
- How much material is recycled?
- What trends exist in these flows and stocks?

A complete MFA/SFA would end up in a full materials (or substance) flow cycle. A great number of such cycles have been described and characterised over the years, particularly for elements (such as carbon and nitrogen) with strong or dominant natural sources and sinks. Less effort has so far been expended on materials whose cycles are dominated by anthropogenic activity. Prominent examples of such exercises carried out in the 1990s were:

- Ayres' (1997) analysis of the life-cycle of chlorine and the chlorine-mercury connection;
- studies by Stigliani et al. (1994) on cadmium in the Rhine basin and by Thomas and Spiro (1994) on the global lead cycle, both studies published in a book on “Industrial Ecology and Global Change” in 1994.

In general, over the last ten years, there has been a growing interest in constructing cycles of metals in defined geographical areas, as the information provided by such exercises is considered useful as a background for the understanding of resource availability and long-term environmental change. It is obvious that the construction of well-quantified metal cycles will be needed if we are to form sound policies related to production, consumption and recycling of metals as well as for taking decisions regarding a sustainable environmental policy. Thus, there are a number of examples of more or less comprehensive metal cycles, such as the MFAs in the City of Stockholm, carried out as a part of the national Swedish research project "Metals in the Urban and Forest Environment” (see further details in sub-chapter 3.3 of this report), and the MFAs, covering the whole Swedish territory, for copper, zinc, chromium, nickel and molybdenum, which were reported in the three previous monographs (L & L – Zn, L & L – Cu, and EW – Cr,Ni,Mo).

With a specific focus on copper, a few additional studies, except those covering Stockholm and Sweden, have been published in the last few years, viz. one dealing with the situation in Japan (Simada et al., 1999), one with the Netherlands (Van der Voet et al., 2000) and two with the USA (CDA, 1999; Zeltner et al., 1999). Recently, a similar approach for copper was tried in a study covering the European territory, defined as the EU together with Poland. The results of this study have been published as a series of four papers under the collective title “The contemporary European copper cycle” in the international journal “Ecological Economics”, (Graedel et al., 2002; Spatari et al., 2002; Bertram et al., 2002; and Rechberger and Graedel, 2002). The stated reason for making this effort was that the previous attempts to study comprehensive cycles for copper did not incorporate all life stages and dissipative flows of copper. Therefore, the group of researchers at Yale University, New Haven, together with colleagues from Cottbus, Germany and Zürich, Switzerland, devised a framework for characterisation of contemporary copper cycles with the following uses in mind:

- To identify the stocks and flows of copper from extraction to end of life, in order to discover opportunities for increasing the efficiency of industrial copper utilisation.
- To evaluate the magnitudes of dissipative flows to the environment.
- To develop information for the consideration of resource policy and environmental policy initiatives (Graedel et al., 2002).

This study is an important further development of the earlier copper cycles, e.g. of the one constructed for Sweden (L & L – Cu). Both the recycling rates and the dissipative flows have now been estimated with greater accuracy, and the present cycles cover a more relevant geographical region, i.e. Europe. Therefore, the main results of the study will be summarised below.

3.1.2 Selection of boundaries of the system to be studied

The goal of the study was to establish the copper cycle over 1 year in contemporary Europe while ensuring that ≥80% of the flows were captured. The year 1994 was chosen as the year of investigation.

According to statistics published by the International Copper Study Group (ICSG, 1999), the 15 Member States of the EU together are responsible for about 90% of the European copper usage, but only for about 25% of the copper production. If Poland, the country having the highest copper production rate in Europe, is included, the system boundary covers 95% of Europe's refined copper usage and 80% of its copper production (Spatari et al., 2002). The chosen system boundaries also ensure that production waste such as slag and tailings, the stock of consumer products in private households and infrastructure, and consumer waste are included. Moreover, the definition is convenient because of the relatively good data availability at the level of the EU. In order to distinguish the system from the traditional definition of Europe, the system was designated as "STAF-Europe".

The model for the copper cycle in the technosphere is made up of four main components or processes:

1. Production, including mining, milling, smelting and refining
2. Fabrication and manufacturing (copper wire, tube, sheet, strip; alloy rod, sheet, strip, etc.)

3. Use (in buildings, industrial machinery, vehicles and infrastructure)
4. Waste management: recovery and recycling, landfilling, losses to the environment.

The STAF-Europe system is connected to the external technospheres through import and export, and to the natural surroundings (the lithosphere and the biosphere).

3.1.3 Some definitions and characterisation of the technical components

The first paper in the the series of four (Graedel et al., 2002) proposes a number of definitions that are needed to agree upon:

Reservoir is a compartment or group of compartments that contains the material of interest.
Stock is the amount of material contained in the reservoir.
Budget is the balance sheet of all inputs and outputs (the *flows*) to a reservoir.
Cycle is a system of two or more connected reservoirs, where a large part of the material is transferred through the system in a cyclic fashion.

Environmental reservoirs (wetlands, sediments, landfills) are usually treated as *accumulative reservoirs*, since geological recycling from these reservoirs only occurs on time scales longer than the span of human societies.

The main components of the technical copper cycling are presented in more detail below:

3.1.3.1. Production (mining, milling, concentration, smelting, refining)

The grade of copper ores that were mined and milled in the early years of the 20th century often was in the range 2-3.5%. However, most of the rich deposits of copper ores have been mined out. Thus, the grade of copper ore in a mining region may sometimes drop below that of gangue left from earlier mining. Today, several big mining companies exploit low-grade copper ores in open pits, some of them extract ore with a grade below 0.5% and even down to around 0.3%. Only in exceptional cases, ores currently being exploited have grades of >4% copper (which is the case for the sulphidic ores from the Storliden mine in northern Sweden), although the size of such rich ore deposits apparently is quite small.

Due to the constant decrease in average grade of copper ores, increasing quantities of tailings (remnants from the concentration process) are being left at mill sites. Since the separation of the valuable mineral from the gangue is imperfect – and was even more imperfect in the beginning of the century – tailings contain copper. Tailings that were dumped by the year 1900 often had an average copper content of 0.75%, while those dumped by the end of the century had less than 0.1% copper. Thus, the improvements in milling methods since the early 20th century have roughly kept pace with the decrease in the grade of ore mined, i.e. the recovery ratio has changed relatively little in the last 80 years (Hancock and Pons, 1999).

In the smelting process, the main loss of copper is with the slag. In the early years of the 20th century, smelter slag typically contained 0.35% copper, and the slag was discarded directly from the furnaces to nearby dumps. The adoption of flash smelting by the end of the century reduced energy consumption, but increased copper concentration in slag. In the 1990s, the copper levels in the slag usually were >0,5%. The ratio of slag discarded to the amount of metal produced depends on the copper content of the concentrate, and this ratio has been relatively constant, about 2.0 on average, during the 20th century (Graedel et al., 2002). Thus, the amount of copper being dumped on landfills together with the slag is not insignificant.

Electrolytic refining generates copper cathodes (the main product), spent electrolyte and anode mud (the material that remains after the anodes are dissolved). Anode mud contains copper, selenium, tellurium and various precious metals, and is usually processed by roasting and leaching to recover these – often valuable – by-products (Graedel et al., 2002).

3.1.3.2. Fabrication, manufacturing and use

Fabricators convert cathode copper into semi-finished products (shapes and ingots), which are shipped to manufacturers. Few of the semi-finished products are ready for use as such, rather they go to first-stages manufacturers, who make metal rods and bars, wires and castings, and then to component manufacturers, who produce things like motors, valves and insulated cables. The components are incorporated by final-stage manufacturers into various finished products.

Metal losses in fabrication and manufacturing become “prompt” scrap that is quickly recycled, mostly internally. Prompt scrap being formed during fabrication of semi-finished products is usually not included in the official statistics, while the prompt scrap being recycled from manufacturers back to fabrication is. Brass and other alloys tend to be recycled in alloy form rather than being reduced to the individual alloying metals. Overall, net losses to waste repositories from fabricators and manufacturers are generally regarded as negligible (Graedel et al., 2002).

A great deal of thinking and discussion has been devoted to the problem of defining the various use categories of copper in the most convenient way to facilitate the copper cycle analysis. The proposal given by the Market Analysis Co., Birmingham, England (Joseph, 1999), includes 17 classes of copper-containing products, uses that accout for 79% of the world-wide consumption of copper, see Table 3.1.

Table 3.1. Principal uses of copper in the world, in 1990 (Joseph, 1999, Graedel et al., 2002).

No:	Category	Use (%)	RT* (years)	S / W**
1	Building wire	14	45	50/50
2	Tube	12	60	45/55
3	Alloy rod	11	20	10/90
4	Magnet wire	9	15	50/50
5	Telecommunication wire	8	50	25/75
6	Power cable	8	40	60/40
7	Copper sheet and strip	8	50	60/40
8	Alloy sheet and strip	7	25	20/80
9	Casting alloys	6	30	50/50
10	Motor vehicle wire	4	10	80/20
11	Appliance wire	4	20	50/50
12	Bare wire	3	10	0/100
13	Copper rod	2	40	60/40
14	Alloy tube	2	35	95/5
15	Wire (other)	1	5	0/100
16	Alloy wire	1	5	5/95
17	Chemical and powder	<1	1	5/95

* estimates of the residence time (RT) of copper in each use;
** estimates of the distribution between scrap (S) and waste (W) at the end of use

Some of the estimates in Table 3.1 could be subjected to discussion. It should also be kept in mind that the distribution of copper uses given in the Table represents the situation in the year 1990. In some of the applications (e.g. in vehicles), there is, of course, a rapid development of the types and the relative importance of uses as well as of the life-times for the different uses. It is clearly necessary to take the great diversity into account when estimating copper stock in usage repositories. However, the classifications and the estimates presented in Table 3.1 seem to be quite useful for the establishment of a credible copper cycle for STAF-Europe.

In order to characterize the in-use stock of copper, the authors (Graedel et al., 2002) have moved from the initial copper-containing products (Table 3.1) and produced a list of seven principal end uses with an indication of the typical amount of copper in each end use. Examples of such end uses are: automobile – 20 kg Cu; residential building – 200 kg Cu; per capita telecom – 4.0 kg Cu; and per capita electricity – 4.3 kg Cu. The typical copper content in commercial and industrial buildings and in industrial machinery is highly building and sector dependent and must be estimated from region to region or from industrial sector to sector.

The in-use stocks were then estimated by setting suitable average copper contents for each of the proxy indicators, multiplying these factors by the quantity of proxy indicators within the region of interest and summing the results. Independent validation has been made where feasible (Graedel et al., 2002).

An important aspect when analysing the buildup and decline of in-use stocks is, of course, a clear understanding of the distribution of service lifetimes of the various copper-containing products. Thus, it is important to estimate not only the total stock of copper, but also the age distribution for metal in service. Any metal older than the average service lifetime in a particular use will become available for reuse and recycling, but the actual reuse / recycling also depends on the physical form of the metal. An illustration of stock buildup and decline was given by Graedel et al. (2002) for copper building wire with an average lifetime of 45 years (range 35-55 years), with a yearly addition to stock of 10 copper units during the use period, and with total use period ending after 55 years. Building wire copper from stock put in place at the start of the period begins to become available for reuse or recycling after 35 years and the stock in service reaches steady-state after 45 years. After the copper building wire becomes obsolete, the stock gradually declines and disappears 55 years after the last installation (Table 3.2).

Table 3.2. Example of stock buildup and decline for copper building wire (see text).

Year	Added to stock	Removed from stock	Net stock
0-5	50	0	50
6-10	50	0	100
- - - - - - - - - - - - - -			
31-35	50	0	350
36-40	50	8	392
41-45	50	25	417
46-50	50	42	425
51-55	50	50	425
56-60	0	50	375
61-65	0	50	325
66-70	0	50	275
- - - - - - - - - - - - - - - - -			

3.1.4 Waste management subsystem

In the project aiming at the establishment of the comtemporary European copper cycle, the waste management subsystem has been thoroughly studied in the third paper, by Bertram et al. (2002). The reader is referred to the original paper to get an account of the sources of the baseline data and of the various assumptions used in the calculations. In the present context, only a brief summary of the main results will be given.

The types of solid waste categories examined in order to get a picture of the copper flows through the waste management system in STAF-Europe, are the following:

- Municipal solid waste (MSW), which shows a relatively low variation between countries, i.e. from 250 kg/cap., year in Austria to 680 kg/cap., year in Luxembourg, with an average of 400 kg/cap., year.
- Construction and demolition waste (C&D), where the waste generation rate is much lower in sparsely populated countries, such as Sweden, Greece and Ireland (<200 kg/cap., year) than in countries with high population densities, e.g. Germany, the Netherlands and Luxembourg (725-750 kg/cap., year).
- Sewage sludge (SS) expressed as dry weight is generated at a yearly rate of 19 kg/cap. on average and varies from <10 kg in Poland and

Belgium to >30 kg in Denmark, partly reflecteing the percentage of the population served by sewage treatment facilities.

- Hazardous waste (HW) generation varies a great deal from country to country with the lowest figure for the UK (41 kg/cap., year) and the highest for Luxembourg and Belgium (358 and 200 kg/cap., year). The average figure for STAF-Europe has been documented at the level of 90 kg/cap., year.
- Industrial waste (IW) includes all wastes from industry and manufacturing that are non-hazardous. (However, it does not include the prompt metal scrap formed at the production units for semi-finished products and goods.) The generation of IW ranges from 40 (Portugal) to about 1600 (Sweden) kg/cap., year, with an average of about 850 kg/cap., year.
- Waste from electrical and electronic equipment (WEEE) is an important waste fraction in the copper cycle, but the real waste generation rate turned out to be very difficult to quantify, which was indicated by the wide gap between the theoretically expected generation rate of 14 kg/cap., year and the actual possible collection rate from private households (4-5 kg/cap., year). This suggests that a significant fraction of retired electronic products remains in “hibernation”, i.e. they are not collected, but rather stored in households, in repair shops or circulate in second-hand markets. Due to this difficulty, an educated guess of the average generation rate, set at 7 kg/cap., year (for all countries except Poland), was used in the calculations.
- End-of-life vehicles (ELV) was quantified as: ELV = □Stock + New + Import – Export , where “□Stock” is previous year’s vehicle stock minus actual vehicle stock; “New” is first registrations of vehicles; “Import” is imports of used vehicles and “Export” is export of used vehicles. With about 9.5 million passenger motor cars, about 1.4 million trucks and 20 000 buses and coaches being retired in 1994 in STAF-Europe, an average copper content of 2% in passenger cars and 0.5% in larger vehicles (trucks, buses), giving a weighted average of copper in cars, buses and trucks of 0.9%, the estimated total amount of copper in ELV for the year 1994 was about 240 kt.

Table 3.3 summarises the waste generation and copper contents for STAF-Europe in 1994 (all abbreviations in the table are explained above):

Table 3.3. Solid waste generation and copper content in the waste for STAF-Europe, in 1994 (after Bertram et al., 2002).

Waste category	Waste generation in kg/cap., year	Waste generation in Mt/year	Waste generation %	Copper content %	Copper content kt/year	Copper fraction %
MSW	400	165	21.5	0.05	83	9.0
C&D	430	184	24.0	0.07	129	14
WEEE	7	3	0.4	13	377	41
ELV	--	27	3.5	0.9	243	26.4
SS	20	8	1.0	0.04	3	0.3
HW	90	30	4.0	0.05	15	1.6
IW	850	350	45.6	0.02	70	7.6
STAF-Europe	410	767	100	0.12	920	100

As can be seen in Table 3.3, the estimated total amount of copper in post-consumer wastes in STAF-Europe in 1994 was about 920 kt. In addition, some 300 kt of copper in imported old scrap entered the waste management system. Roughly 60% of the copper scrap used in European secondary copper production comes from prompt manufacturing scrap (980 kt/year) and about 40% (740 kt/year) from old scrap. The 740 kt is made up of 300 kt imported and 440 kt domestic scrap. This means that about 48% of the domestically generated copper waste is recycled, while the remaining 52% is either landfilled (about 480 kt/year) or lost to the environment. Calculated on the total amount of post-consumer copper waste (1220 kt/year), the recycled fraction is more than 60%.

The dominant part of copper scrap used in secondary production of copper is new or prompt scrap generated within the large fabrication and manufacturing base in Europe. Only about 26% of the scrap used in domestic secondary production comes from old non-imported scrap. About 55% of the copper scrap is re-melted directly by brass mills, wire rod producers, foundries and ingot producers, the remaining 45% goes back to the smelting and refining processes (Bertram et al., 2002).

In Table 3.3 is also shown the relative importance of copper in the various waste categories. WEEE, ELV and C&D are by far the most relevant waste categories in terms of their potential for copper recovery. WEEE, which has a rapidly growing generation rate, is a particularly attractive future resource for secondary copper. A more detailed analysis of the present rate of recovery and recycling of copper from the three mentioned waste categories, and of their future potentials may be of interest:

- WEEE is one of the most complex waste streams, and there is a general lack of adequate treatment facilities for this waste. Though mechanical separation standards make it possible to recycle 80-90% of WEEE, the cost of recycling most types of electronic equipment is higher than the value of the recovered metal (Langer, 1994). A significant proportion of WEEE in Europe is probably still disposed of as part of the municipal waste stream, stored in households or illegally dumped. However, increased efforts for recycling of WEEE have been initiated lately, and the recycling efficiency of copper in WEEE is currently supposed to be about 50%. In this context, it may be mentioned that the Swedish base metal producing company Boliden AB recently considerably increased its use of WEEE as a raw material in the secondary production of copper (Sundberg, pers. comm.).
- ELVs have a great potential as a secondary copper resource. According to a study by Frei et al. (1993) of vehicle scrapping plants, only about 40% of the copper contained in automobiles reached the scrap market in the early 1990s. Later on, Russo et al. (1999) found that dismantling steps prior to shredding make it possible to separate 90% of the copper in ELV during the disposal procedures. Currently, it is assumed that 70% of the copper in the ELV stream is recovered and recycled (Bertram et al., 2002). According to a recent EU Directive, 85% of a scrapped automobile should be reused or recycled, before January 1st, 2006.
- C&D recycling was examined in an EU study (1999), and it was found that recycling and reuse of C&D varied from <5% in some countries (e.g. Spain, Portugal, Greece and Ireland) to 90% for others (the Netherlands), resulting in a weighted average of 28% for Europe. However, most of the countries with poor recycling infrastructures have an active informal recycling industry, based on hand collection of all items with an economic value (e.g. copper tubes) before the C&D is landfilled. Other studies (e.g. Brunner and Stämpfli, 1993) have shown that up to 69% of copper leaves a waste sorting plant with the metal fraction, which can theoretically be recycled. About 50% of the copper was separated by hand sorting. Based on the different information sources available, Bertram et al. (2002) concluded that the recycling efficiency for copper in C&D in Europe would be between 30 and 50%.

The overall conclusions from the study of copper in the waste management system in STAF-Europe (Bertram et al., 2002) may be summarised as follows:

- About 2 kg per capita of copper waste is generated annually in the region.
- WEEE and ELV are the two domestic waste streams with the highest copper content; they contain 67% of the total copper throughput, but only make up 4% of the total waste mass.
- WEEE is the fastest growing waste category (annual growth rate of 5-10%), a fact that emphasizes the need for efficient WEEE recycling strategies.
- Overall recycling efficiency in the region for copper in all types of waste (excluding prompt scrap and scrap imports) is 48%, with a range of 5-58% depending on the country.
- Consequently, there is further potential for increased recycling activities in the future.
- For example, it is possible that separate collection and mechanical dismantling of WEEE is superior to a system where consumer appliances and MSW are collected together and sent to an incinerator with adjacent recovery of copper from the bottom ash.
- Although the copper content of MSW, SS, IW and HW is small and their relevance for copper recovery is not significant, efforts should be made to reduce the loss of copper via these waste categories.
- The distribution pattern of copper in the waste management system shows that high recovery rates are theoretically possible by applying appropriate technology to selected waste streams.
- In general terms, it is much easier and quicker to undertake such changes, rather than introducing structural changes such as the redesign of industrial and commercial products.

3.1.5 Summary of stocks and flows of copper in European society, in 1994

The second paper in the series dealing with the contemporary European copper cycle proposes a complete copper cycle for STAF-Europe (Spatari et al., 2002).

The authors have examined the major flows of copper from ore, copper transformed into products, and discarded or recycled. Successive mass balance calculations were conducted for the different main processes:

production, fabrication / manufacturing and waste management, in order to determine copper flows as well as the amounts of metal that enters use in society and is deposited in waste repositories.

The majority of copper used in the region is mined, smelted and refined outside of Europe, meaning that a net total of 1,900 kt of copper was imported into Europe in 1994. To supply the copper smelting industry in STAF-Europe, about 600 kt of copper originated from ore mined within the region (690 kt of copper in the concentrates and 90 kt in the tailings, which go to landfilling) and 280 kt contained in imported concentrates. About 12 kt copper in smelter slag was deposited in landfills.

Refineries are supplied with copper from the smelters, but to a large extent also with imported blister copper and with post-consumer, old scrap as well as with new scrap from the fabrication / manufacturing sector. Also the latter sector takes a considerable share of its raw material (cathode copper) from imports (about 1,300 kt/year), while cathod copper from domestic smelters accounts for some 2,300 kt/year, and recycled old scrap for about 200 kt/year. In addition, there are significant internal loops of scrap, e.g. between the manufacturing and fabrication operations. The output of non-alloyed copper products is to a dominant part used domestically (2,650 kt/year), since only about 70 kt/year is exported. As far as the finished copper alloy products are concerned, about 88% are used domestically (780 kt/year). Thus, the majority of copper in finished products is contained in pure form (75%).

Since the amount of copper entering the waste management system is about 920 kt/year (see 3.1.4), the rate of yearly increase of the stock of copper in European society is about 2,500 kt (2,650 + 780 – 920 ~ 2,500 kt). Calculated on a per capita basis, the net addition of copper to the stock in society in the region is 6 kg/year (Spatari et al., 2002). The amount of metal in waste repositories – landfills, including tailings ponds – also grows by a yearly rate of about 580 kt, corresponding to a growth rate of 1.4 kg/capita.

The information from the different steps in the copper cycle was then integrated to provide a comprehensive continental-level copper cycle (see Figure 3:1).

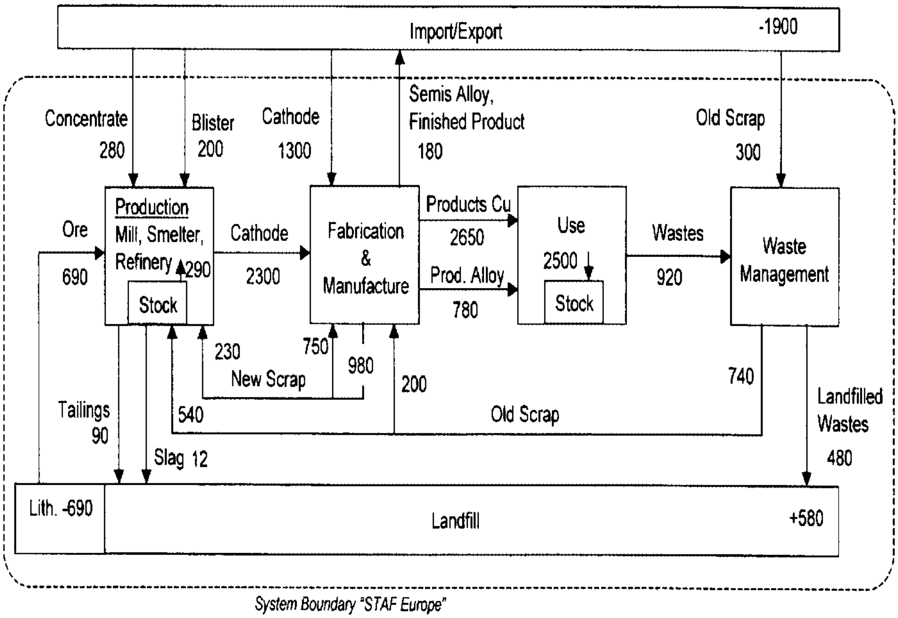


Figure 3.1 The copper cycle for Europe in 1994. All units measured in kt/year. Reprinted from Spatari et al., 2002, with permission from Elsevier

3.2 Example 2: Dynamic modelling of metal flows in the Netherlands; Cu and Zn

In the Netherlands, two mathematical models, "FLUX" and "Dynabox", were used to evaluate options for overall Dutch metals management and the results were published in a book (by Kluwer Acad. Publ.) receiving wide international attention. The first step of this work was to make inventories of flows and stocks of Cd, Cu, Pb and Zn in the Dutch economy. The second step consisted of an analysis of the ultimate health and environmental consequences of the present metal management regime, while the third step was a comparison (expressed in terms of "sustainability indicators") with some possible alternative regimes.

It was found that emissions of all the investigated metals had decreased considerably and are today small compared to the flows through the economy. Although the metal accumulation in the Dutch economy does not cause risk levels to be transgressed at present, the authors claim that with the present management regime, emissions will rise again in the future, causing transgression of human and ecosystem health standards, especially for Cu and Zn (but – surprisingly enough – not for Cd and Pb).

A critical review of the Dutch modelling work and of the conclusions reached, however, results in a great number of questions: Just as one example, there is an obvious contradiction between the statement that the metal emissions to the Dutch environment are low and the figures given to substantiate this. According to data in the book, the emissions of Cu to the Dutch environment would be about 3,500 times higher than those in Sweden and the Zn emissions about 1,600 times higher. Many of the other exercises with numbers shown in the book clearly indicate that the conclusions based thereupon have a low credibility.

3.2.1 Models used

As a part of an interdisciplinary research programme, "The Metals Programme", financed by the Dutch National Science Foundation, two mathematical models have been used to evaluate options for overall Dutch metals management: "FLUX" and "Dynabox". Both are essentially "substance flow analysis" (SFA) models (van der Voet et al., 2000). "FLUX" was used:

- to make an inventory of flows and stocks of cadmium, copper, lead and zinc in, out and through the economy for the base year 1990;
- to calculate steady-state societal flows and stocks, the 'ultimate consequence of the present metal management regime';
- to compare alternative regimes with the present regime on a steady-state basis.

The outcomes of "FLUX" were then translated into sustainability indicators regarding the metal's metabolism. These indicators included some describing the fate of the metals, such as total emissions, total landfill, accumulation in the economy, pollution export, while others were used for evaluation of the present management regime, for example, environmental concentration (Predicted Environmental Concentration divided by Predicted No-Effect Concentration, or PEC/PNEC), human intake (Predicted Daily Intake divided by Tolerable Daily Intake, or PDI/TDI) or environmental accumulation, and a few indicators, finally, for designing a future sustainable management regime, like technical efficiency and recycling rate.

It was recognized that the concept "indicator" is not strictly defined, and in practice many widely differing things may serve as indicators. The indicator concept developed during the Dutch research programme was designed to provide information with regard to:

- the existence and causes of environmental problems related to metals;
- the management of metal chains or cycles in society;
- an early recognition of future problems;
- the influence of policy measures, including both their effectiveness and various types of problem-shifting.

These demands imply the need for defining *reference values* which indicate a desired or sustainable level for the individual indicators. However, for several of the indicators, it was considered difficult or even impossible to define a reference value, which may have some repercussions on the evaluation of the results in terms of future metal flows.

The second model, "Dynabox", was used to calculate substance flows and stocks related to environmental compartments based on physico-chemical substance characteristics. It also contained a risk assessment module translating the flows and stocks into environmental concentrations

and human intake. It also compared these to policy standards or no-effect levels.
The "Dynabox" model was used for the following purposes:

- to calculate steady-state environmental flows and stocks, 'the ultimate consequence of the present metal management regime';
- to calculate the ecosystem and human health risk ratios occurring in the steady state;
- to compare alternative management regimes with the present one on a steady-state basis.

3.2.2 Summary of main results

Based on the primary results obtained by the models, it was concluded that:

- Over the past few decades, emissions of copper, zinc, lead and cadmium to air and water have decreased considerably. Today, the emissions of all four metals are very small compared to the flows through the economy. The inflow of these metals into the Dutch economy has remained at the same level. Instead of being emitted, these metals now accumulate in the Dutch economy in a number of stocks of products and in a number of closed loops connected, in particular, with agriculture.
- The accumulation does not cause risk levels to be transgressed at present. However, the authors claim that if the present management regime continues to be pursued, emissions will rise again in the future, owing to the increased stocks, causing transgression of human and ecosystem health standards, especially for copper and zinc.
- For copper, zinc and lead, a significant "back-flow" can be observed, indicating that a large fraction of the waste materials is recycled. For cadmium, this is not the case: waste materials either end up in landfills or are re-used as building and road construction materials containing cadmium as a trace contaminant.
- For lead, the problems can be expected to disappear owing to the penetration of lead-free gasoline since 1990.
- Surprisingly, cadmium concentrations appear to remain within the standard limits, both at present and in the steady-state situation.
- For copper and zinc, however, the authors do not find that there are any signs of these problems to be resolved in the future.

The main problem for copper and zinc was found to be related to agricultural soil and aquatic ecosystems. Under the present management regime, it was concluded that large-scale transgression of health standards may be expected roughly within a decade for aquatic ecosystems and within a century for agricultural soils. In addition, it was assumed that the quantity of metals entering the waste stage may pose a management problem: although recycling is a good option for many applications, processing large amounts of copper and zinc gives rise to a considerable volume of metal-containing waste-treatment residues which eventually leach into the environment. In the steady state, both waste generation and leaching of copper and zinc from landfill sites was found to be significantly greater than in the 1990 situation (van der Voet and van Oers, 2000).

Table 3.4. Origins of the immission of copper and zinc in surface waters in the Netherlands. S.S. stands for a steady-state scenario. After van der Voet and van Oers, 2000.

Origins	Cu (1990)	Cu (S.S)	Zn (1990)	Zn(S.S)
Total immission, t/y	570	1,050	2,78	3,580
Transboundary inflow	68%	37%	73%	56%
Effluent, sewage treatment	12%	21%	5%	4%
Anti-fouling treatment	9%	5%	-	-
Corrosion from building materials	-	-	10%	24%
Industrial emissions	3%	3%	4%	3%
Dissipative applications	2%	17%	3%	2%
Atmospheric deposition	2%	1%	2%	2%
Runoff from soils	3%	16%	3%	8%
Runoff from landfill sites	-	“very high”	-	“very high”

For aquatic ecosystems, the main source of both copper and zinc pollution is the transboundary inflow via the rivers Rhine and Meuse: in 1990, some 68% of the total copper immission in surface waters of the Netherlands (570 t/y) and about 73% of the total zinc immission (2,780 t/y) was caused by transboundary inflow. In the case of copper in 1990, other noteworthy contributions were from sewage treatment effluents (12%) and antifouling treatments of ships (9%), while other significant contributions to the zinc immission in 1990 were via corrosion of building materials (10%) and sewage (5%), see Table 3.4.

In the steady-state situation, with a much higher immission into surface waters (1,050 t/y of copper and >3,580 t/y of zinc) the transboundary inflow appears to lose some of its importance (37% and 56%, respectively). For copper, the most important increases (compared to the situation in 1990) were for “dissipative applications” (17% of total immission), runoff from soils and landfill sites, as well as sewage effluents. The latter is supposed to depend on an expected substantial increase in the corrosion of copper water pipes. Considerable increases in zinc immission were estimated to occur for corrosion of building material (24%) as well as for runoff from soils and landfill sites. Agriculture is a minor source for both copper and zinc immision in 1990, but in the steady-state situation, its contribution is supposed to have increased by a factor of 4. The only industrial source showing a marked increase between the 1990 and the steady-state situation is the food industry, due to higher copper and zinc contents in agricultural products. For surface waters, however, this remains a minor source.

The authors found that since transboundary inflow from foreign countries cannot be regulated by the Dutch policy, it is even more important to address the following domestic sources:

- copper water pipes
- anti-fouling treatment of ships with copper-containing materials
- zinc building materials or building materials coated with zinc
- emissions from landfill sites (especially in the long term)
- agricultural flows of both copper and zinc (in the long term).

The dominating origin of copper and zinc in agricultural soils is animal manure (79% of the total immission of 1,020 t/y for copper and 79% of the total zinc immission of 2,290 t/y, in both cases for the year 1990) and this origin will become even more dominant (82% and 86%, respectively) in the steady-state situation, Table 3.5.

Table 3.5. Origins of the immision of copper and zinc in agricultural soils in the Netherlands. S.S. stands for a steady-state scenario. After van der Voet and van Oers, 2000.

Origins	Cu (1990)	Cu (S.S)	Zn (1990)	Zn (S.S)
Total immission, t/y	1,020	1,960	2,290	3,420
Animal manure	79%	82%	79%	86%
Phosphate fertiliser	11%	6%	1%	1%
Compost	6%	10%	4%	3%
Pesticides	1%	-	3%	2%
Atmospheric deposition	4%	2%	13%	9%

The predominance as well as the increase of this origin for copper and zinc is explained by the fact that animal manure is part of a closed loop and the main addition from outside to this loop (animals eat fodder, which is grown with manure, which is produced by animals eating fodder, etc.) is copper and zinc intentionally added to the fodder for productivity reasons. The authors point out that such a closed loop implies that even with relatively small additions – and the additions are indeed small compared to other zinc and copper flows – environmental stocks may rise above health risk standards. For a further discussion of the copper and zinc balances in agricultural soils, the reader is referred to sections 4.2.4 and 4.2.5 of this report.

When it comes to the most important origins of copper and zinc in the waste management system, 90% of the total amount of waste copper generated in the Netherlands (50,400 t/y) and 76% of the zinc waste (42,400 t/y) originates from building and demolition waste, Table 3.6. This fraction will decrease for copper (to 80%) but increase (to 59,200) in absolute number in the steady-state situation, and will increase for zinc both in relative (to 82%) and absolute numbers (to 53,800 t/y). Consumer applications contribute by <10% for the two metals both in 1990 and in a steady-state scenario, while industrial sources are important only in the case of zinc (13 and 8%, respectively).

Table 3.6. Origins of copper and zinc in the waste management system in the Netherlands. S.S. stands for a steady-state scenario. After van der Voet and van Oers, 2000.

Origins	Cu (1990)	Cu (S.S)	Zn(1990)	Zn (S.S)
Total waste generated in NL*, t/y	50,400	74,000	42,400	65,600
Building and demolition waste	90%	80%	76%	82%
Contaminant in concrete/iron & steel	1%	4%	1%	-
Discarded consumer applications	8%	9%	9%	7%
Industrial waste	-	-	13%	8%
Other waste materials	-	6%	1%	2%

* NL = The Netherlands

3.2.3 Critical review of the Dutch calculations

Many of the estimated metal fluxes, metal risk ratios and other quantitative data presented in the book, “Heavy Metals: A Problem Solved?”, edited by Ester van der Voet, Jeroen B. Guinée and Helias A. Udo de Haes (2000) are quite surprising, to say the least. Since the book, published by Kluwer Academic Publishers may have a strong impact on the philosophy and on the decisions to be taken by environmental authorities and politicians in many European countries, it is necessary to take the data presented seriously and subject them to a careful scrutiny, in order to assess the credibility of both the numerical results of the modelling and the general conclusions reached by the authors.

In section III.1 of the book, the authors conclude that “in most cases emissions constitute only a small part of the total fate of the metals. This does not imply that these emissions cause no problems: metals are toxic even in small doses.” However, the quantitative data on emissions of metals in the Netherlands presented in Figure III.1.3 in the book clearly indicate that the emissions would be extremely high; in fact, if the data were correct, there is no question that a very serious metal contamination of the Dutch environment is existing. The data given in Table 3.7 can be extracted from Figure III.1.3 in the Dutch book.

Table 3.7. Total emissions of metals to the Dutch environment in 1990 and at a steady-state scenario, as well as the main receiving compartment, according to data in van der Voet, Guinée and de Haes, 2000.

Metal	Total emission in 1990	Tot. emis. at steady state	Main receiving compartm.
Cu	1,250 ktonnes	2,500 ktonnes	Agr. soils (1,900 ktonnes)
Zn	5,300 ktonnes	11,900 ktonnes	Non-agr. soils (7,500 kt)
Pb	1,150 ktonnes	1,400 ktonnes	Agr. + non-agr. soils
Cd	15 tonnes	22 tonnes	Agr. soils (10 tonnes)

Just for comparison with the metal emissions in another European country with approximately the same population, Sweden, it can be mentioned that the total emissions of copper to the Swedish environment in the mid-1990s were estimated to a little more than 200 tonnes, of which somewhat more than 100 tonnes were emitted to the aquatic environment (L

& L – Cu). In addition to the 200 tonnes to the natural environment, some 150 tonnes were introduced to agricultural soils. Thus, the total of about 350 tonnes of copper "emitted" in Sweden constitute less than 0.03% of the reported emissions in the Netherlands.

A similar comparison for zinc, based on data from (L & L – Zn), results in a fraction of about 0.06% as the Swedish emissions related to the Dutch emissions. Only the figures for cadmium seem to be in a reasonable order of magnitude.

The authors explain the great differences between the 1990 data and the steady-state data in the following way:

"The increase of steady-state emissions to agricultural soils compared to 1990 emissions is significant for all metals and is due to increasing flows of organic manure and of source-separated vegetable, fruit and garden waste (. . .). The ultimate source behind these increasing flows of copper and zinc is animal fodder."

In their evaluation of the present metal management policy in terms of sustainability, the authors have used indicators for environmental concentrations (PEC/PNEC ratios), for human intake (PDI/TDI ratios) and for environmental accumulation both in 1990 and in a steady-state scenario. In order to assess the level of the PEC/PNEC ratios, i.e. calculating the risk ratio for aquatic and terrestrial ecotoxicity, the Dutch Maximum Permissible Concentration (MPC) values have been applied (these values were taken from references either in Dutch language or a not readily available RIVM report). However, the MPC values, as shown in Figures III.1.5 and III.1.6, are equal to 1, i.e. the limit value for the PEC/PNEC ratio, above which an unacceptable risk for the ecosystem might be at hand. Usually the MPC value is not expressed as a ratio, but as a concentration in absolute numbers. Therefore, it is far from obvious what is meant by MPC in the present context. Maybe it is meant that the MPC value is equal to the PNEC value, but no PNEC value is given in clear number for any of the metals. Therefore, it is impossible to make a proper assessment of the validity of the reasoning by the authors. This ambiguity does not prevent the authors from using the (unspecified) MPC values in the evaluation.

The evaluation has resulted in the following conclusion: "*At present, MPC values are not transgressed for any of the metals. However, in the steady state ecotoxicological risk ratios are expected to be over 1 for all metals except cadmium.*" In fact, from Figure III.1.5 it can be seen that the PEC/PNEC ratio for copper in the aquatic environment will increase from about 0.2 to 35.0 when we move from the 1990 situation to the steady-state scenario. The terrestrial ecotoxicity risk ratio for copper will rise from about 0.7 in 1990 to 6.7 at the steady-state situation. Again, the ratio for cadmium will increase just a little when moving from the 1990 to the steady-state situation, but never transgress the limit value of 1.0. If we assume that the PNEC value (or possibly the MPC value) for copper in the aquatic ecosystem will remain the same in the year 1990 and at a steady-state scenario, then the dramatic rise of the ratio from 0.2 to 35, i.e. rise by 175 times, must depend on an increase in the PEC value by 175 times. It is hard to understand how the doubling of total copper immission (cf. Table 3.4), or the doubling of total copper emissions (in fact an increase by 5 times in the emissions to water was calculated by the authors) from 1990 to the steady-state situation can cause a 175-fold rise in PEC. Unfortunately, it must be concluded that the data presented in the book under review simply do not stick together.

It is also interesting to note that the accumulation of copper and zinc in the environment is considered to be so fast that the transition period for risk ratios in the Dutch environment to become unacceptable is 3 and 16 years, counted from 1990, for copper and zinc, respectively, in the aquatic environment, while it is 1000 years for lead and the transition period for cadmium is eternal. These conclusions by van der Voet, Guinée and de Haes (2000) on the future environmental and health risks of copper and zinc, compared to the risk of cadmium, must be considered to have a low credibility.

3.3 Example 3: Urban metal flows – Stockholm; Cr, Cu, Ni and Zn

After the preparation (by MFG) of the three monographs on metals in society and the environment in Sweden, in which preliminary mass balances of Cr, Cu, Ni and Zn were included, more detailed data was compiled on metal stocks and fluxes in the city of Stockholm and comprehensive mass balances for metals were established for the Stockholm area. This work, published in 2001, was part of the project "Metals in the Urban and Forest Environment", financed by the Swedish EPA.

The total stock of Cu in Stockholm was slightly greater than 120 kt with an annual net growth rate of about 1.6%. The stock of Zn was less than a fourth of the Cu stock (28 kt) with a net growth of 4.3%. Ni showed that fastest growth (6.4%), but from a low stock level (2.5 kt).

Metal emissions, except for the flows to the solid waste compartment, were twice as high for Zn (24 t/y) as for Cu (12 t/y), while known emissions of Cr and Ni were low (0.8 and 0.6 t/y). Considerable fractions of these amounts of metals are channelled through sewage treatment plants, where most of the incoming mass of metals is incorporated into the sludge. However, a substantial part of the total releases of metals to water from the city of Stockholm ends up in the Stockholm Stream: compared to the large-scale flux of metals from Lake Mälaren to the Baltic Sea, the contribution from Stockholm city is 15-25% for Cu and Ni, 30-40% for Cr and 40-55% for Zn.

The types of goods causing the greatest releases of metals in Stockholm were found to be:

- *for Cu: water pipes in buildings, followed by motor vehicle brakes;*
- *for Zn: motor vehicle tyres, followed by various galvanized materials;*
- *for Cr and Ni: road pavements, followed by tyres.*

Based on a comparison between the estimated metal emissions from various goods and the measured quantities of metals entering the biggest sewage treatment plant in Stockholm, it was concluded that virtually all existing sources of Zn had been identified, while the total emissions of Cu from possible sources were somewhat overestimated. By contrast, the correspondence between measured quantities and estimated emissions was poor to very poor for Ni and Cr. A closer look at the estimates of Zn and Cu emissions, however, tells us that also for these metals, there is a need to revise the figures published. In particular, it was found that a better correspondence between total fluxes from identified sources and measured inflows to sewage treatment plants can be obtained, if a certain retention in soil and concrete of Cu and Zn released from roofs and other infrastructure materials is taken into account.

The recently published material also allows a more accurate picture to be drawn of the pools and fluxes of the trace metals in the groundwater, soils and lake sediments in Stockholm. The total storage of metals in the aquifer of Stockholm was estimated at about 2.5 t for Zn, 0.7 t for Cu, 0.6 t for Ni, while less than 70 kg of Cr were stored in the groundwater. The total fluxes of the same metals with groundwater were estimated at 0.41 t, 0.12 t, 0.09 t and 0.01 t, respectively. If the entire metal transport with groundwater in Stockholm is compared to the total flux of metals from the Stockholm anthroposphere to the biosphere, the groundwater fraction constitutes 0.8-1.8%. In fact, metal fluxes with the Stockholm groundwater are much smaller than those in forest ecosystems, due to lower groundwater recharge in urban areas.

The comprehensive studies of metals in urban soils in Stockholm showed that the mean levels of Cu and Zn were enhanced 2-3 times in the city centre, compared to reference levels in regional arable soils and undisturbed soils within the city. No enhancement was found for Cr and Ni. The only soils showing considerably increased levels of Cu and Zn were those where former industrial activities had taken place. Here, 10-15 times higher levels of Cu and about 5 times higher levels of Zn, compared to reference values, were recorded.

Investigations of metals in the aquatic sediments in the Stockholm area, carried out as part of the programme "Metals in the Urban and Forest Environment", have been used to estimate the relative contribution from the city to the annual load of metals on the sediments. It was found that Stockholm every year contributed about 1 t of Cr, 1.7 t of Cu, 4 t of Zn, but no Ni to the regional aquatic sediments. Compared to the total fluxes from the city, via sewage treatment plants, storm water and groundwater, 0.4 t/y of Cr, 2.6 t/y of Cu, 9.1 t/y of Zn and 1.5 t/y of Ni, it might be concluded that there must be some still unidentified sources of Cr in the city, while a relatively small amount of Cu, (2.6 – 1.7) = 0.9 t/y, escapes deposition in the innermost archipelago and large to very large relative amounts of Zn, (9.1 – 4.0) = 5.1 t/y, and Ni are transported further out across the archipelago into the Baltic Sea.

3.3.1 New aspects of studies on urban metal flows

Mass balances of various substances or materials, such as metals, usually have been established for large areas, e.g. countries or regions, like the European Union or the United States of America. An example of such an exercise – for copper in the EU + Poland – was presented in section 3.1. A good justification for this broad defininition of the boundaries of the system to be analysed is that both mining, primary production and secondary production units are present within the boundaries, together with the multitude of consumption sites. However, studies where the system is restricted to a smaller geographical area, e.g. to a single city, may have the advantage of being able to emphasize specific aspects, such as:

- the role and relative significance of diffuse sources of metal dispersion to the natural environment;
- the transformation and speciation of metals during transport from sources in society to the end depositories: and
- the possible exposure of sensitive environmental compartments to bioavailable species of metals.

In earlier studies of urban metal flows, the main focus was on analyses of flows of solid waste, storm water and waste-water. (Davidsson, 1978; Malmqvist, 1983). In later years, calculations of mass balances of metals in society have usually been initiated by authorities, either responsible for the sustainable use of materials in connection with sound management of natural resources or dealing with assessments of the environmental and health impacts of metals used in various applications.

In order to assess the impacts of urban metal flows on society's metal recycling systems and on the environment, a five-year research programme was launched in 1994 by the Swedish Environmental Protection Agency (SEPA, 1994) under the name of "Metals in the Urban and Forest Environment". The system boundaries of the study were the administrative border of Stockholm City, representing an area of 190 km^2 with approximately 700,000 inhabitants. The study comprised flow analyses for the year 1995 of seven metals, in addition to the four metals (chromium, copper, nickel and zinc) treated in this report, also cadmium, lead and mercury were examined.

Industrial handling of metals was quite extensive in Stockholm in the beginning of the 20th century. At that time, the city had a diversified industry consisting of e.g. tanneries, foundries, battery and rubber factories, impregnation and galvanisation plants, gas, dye and printing works (Bergbäck et al., 2001) – all with handling of metals and, consequently, with considerable metal emissions to the surrounding environment. Today, metal-emitting industries have almost disappeared from the city of Stockholm, the economy of which is now dominated by the service, trade and financial sectors, in addition to activities related to the central administration of the country. The city is located on the very short river, Norrström, connecting Lake Mälaren (Sweden's third largest lake) with the Baltic Sea.

3.3.2 Stock of metals in Stockholm

The present metal stock in the city of Stockholm, in infrastructure, buildings, vehicles, etc. has developed mainly during the 20th century, but the use of copper for roofing and cladding of buildings and bronze in statues and other objects of art is a very old practice. According to Lohm et al. (1997), electrification (increasing the stock of copper) of the city started at the turn of the century and culminated in 1920-1960; the tapwater system changed in the 1950s basically from iron and zinc piping to copper pipes; applications of stainless steel (Cr and Ni) and galvanised steel (Zn) became common after the Second World War.

Bergbäck et al. (2001) have calculated the metal stock in the anthroposphere of Stockholm for 1995, together with the different exposure conditions of the metals, according to Sörme et al., (2001a), as well as the annual inflows and the outflows from the existing stock of each metal as solid waste and via other routes, Table 3.8. The outflows are defined as metal fluxes from the Stockholm metal stock, either to the "waste" compartment or by means of "goods emissions" or industrial emissions to any of the other three "end stations" considered, sediment, soil or sewage sludge. The goods emissions are further specified in Table 3.9. The outflows from the anthroposphere to the biosphere, as presented by Bergbäck et al. (2001), also included atmospheric deposition and fluxes via food (including drinking water), but since these two fluxes are not directly related to the metal stock in Stockholm, they are not considered as part of a stock modifying mechanism in the following discussion.

Table 3.8. Stock of metals (ktonnes) in the city of Stockholm, the annual amounts of metals in inflows and outflows – with solid waste and via other routes (ktonnes/y) – and the percentages of metals in stock that are protected and exposed to soil, water and air, respectively. After Bergbäck et al., 2001; Sörme et al., 2001a.

			Percent				Outflow via:	
Metal	Inflow	Stock	Protected	Exp.soil	Water	Air	Sol. waste	Other routes
Cr	0.36	5.6	54	10	11	25	0.1	0.001
Cu	2.30	123	63	14	15	8	0.3	0.012
Ni	0.19	2.5	71	0	9	20	0.03	0.001
Zn	1.9	28	31	0	31	38	0.7	0.024

Table 3.8 clearly shows, first, that the copper stock is by far the biggest of the studied metal stocks and, secondly, that all the four metal stocks were increasing in size at the middle of the 1990s. It was emphasized by the authors (Bergbäck et al., 2001) that there is a relatively wide range of uncertainty in the figures for both existing stocks and outflows with solid waste. Nonetheless, it might be possible to give a broad indication of the rate of change in the stock of the four metals by calculating the percent annual net increase. Thus, it turns out that the nickel stock seems to undergo the fastest increase with 6.4% per year, the zinc and chromium stocks have an intermediate rate of growth (4.3% and 4.6%), while the lowest growth rate was found for the copper stock (1.6% per year).

3.3.3 Outflows of metals from existing stocks to the solid waste compartment

The quantities of metals flowing out with solid waste from each of the stocks of the four metals are rather uncertain. It should also be noted that these quantities leaving the metal stocks in Stockholm include scrap going to recovery and recycling, and should therefore not in reality be labelled “waste”. However, within the borders of the city of Stockholm there are relatively limited facilities for such metal recovery and recycling, and therefore, the “waste” is considered as a quantity leaving the system. From the waste compartment as such the losses of metals to the biosphere are very small, e.g. from incineration and with leachates from landfills.

3.3.4 Outflows via other routes, e.g. diffuse emissions from goods

The outflows via other routes, such as industrial and goods emissions, where the goods emissions are largely predominating, have been determined with greater certainty. In this case, it should be noted, however, that these emissions from the anthroposphere are not necessarily ending up in the biosphere. Thus, the metal amounts that eventually will take part in the exposure of sensitive ecosystems only constitute a small fraction of the reported outflows. We shall first examine the outflows from the major goods emission sources in Stockholm, according to data given by Bergbäck et al. (2001) and by Sörme et al. (2001b), see Table 3.9.

The metal fluxes from the road traffic sector (tyres, brakes, petrol och road pavement), especially if car wash is included, are the predominant emissions from goods in Stockholm, at least for chromium and nickel (about 99% of the total), and also for zinc (62%) but less for copper (38%).With the exception of the source “car wash”, the metals being released from road traffic activities are primarily spread along the roadsides, where the mainly particle-bound metals are incorporated into the soil and largely immobilized (cf. Gartiser et al., 2003).

Table 3.9. Calculated metal emissions (kg/y) from the major goods emission sources in Stockholm, 1995. After Bergbäck et al., 2001 and Sörme et al., 2001b.

Category	Goods	Chromium	Copper	Nickel	Zinc
Vehicles	Tyres	200	200*	200	10,000
	Brakes	6	3,900	8	900
	Petrol	<1	2	<3	60
	Car wash	60	(300?)**	55	3,000
	Boats (paints, anodes)	--	700	--	1,500
Buildings	Roofs/fronts	<1	1,000	0.5	2,100
	Pipes (tap water, etc.)	4	4,300	2	700
	Electrical grounding	-	<900	-	--
Infrastructure	Road pavements	500	400	300	1,000
	Aerial lines, galv.goods	--	1,200	--	5,000
Total emissions		770 +	12,000 +	570	24,000 +

* according to recent information from tyre producers, very low Cu content is expected in tyres
** car wash contribution to total Cu load taken from later estimate, see Table 3.12

In a recent experiment, where the fate and effect of zinc from tire debris was investigated in roadside soils, it was found that a considerable fraction of zinc was released from the rubber matrix within 1 year, but the parallel increase in soil pH counteracts the increase in quantity of labile zinc in the soil. Thus, no or only marginal increase in zinc levels in pore water or in soil leachates was observed in soils treated with tire debris, and the nitrification rate of the soil was stimulated (Smolders and Degryse, 2002).

In highly urbanized areas with no soil strips along roads, a considerable (however, unknown) fraction of the metals released from road traffic is transported with the surface runoff to the stormwater collectors. From there, in parts of the city with combined sewage and stormwater systems, metals are transported to the sewage treatment plants (STP), where a major fraction is separated and incorporated with the sludge, which in turn is disposed of either on a landfill or on agricultural land. The same fate is waiting for the metals (mainly copper and zinc) being released from tap water and other pipes, and partly also for metals in the runoff from roofs and fronts of buildings. These fluxes will be further described in sections 4.1 and 4.2.

3.3.5 Metal fluxes to and from sewage treatment plants in Stockholm

In order to get a general overview of the metal fluxes to and from the STPs in Stockholm, as well as their relative significance compared to, for example, the total metal flow from Lake Mälaren through the Stockholm Stream to the Baltic Sea (according to Lindström et al., 2001), or the total emissions of the same metal from the metal stock, a compilation of data from Bergbäck et al. (2001) has been made i Table 3.10.

Metal fluxes to the water recipient (A) are greater than the difference between the influx to the STPs and the amount removed to the sludge, because of the portion of stormwater that is discharged directly to the water recpient and the minor metal fluxes with groundwater (see Aastrup and Thunholm, 2001). According to Bergbäck et al. (2001), the percentage of metals removed to sludge was 80-90% for chromium and copper, but only 67% for zinc. The removal efficiency for nickel cannot be calculated from the data, because some nickel input with chemicals used in the STP disturbs the picture. It is questionable to regard the metal fractions ending up in sewage sludge, which is either recycled in the farming system (by all standards a man-made, artificial system) or disposed of in landfills, as being emitted to the biosphere (the natural ecosystem). This should relate only to the metal fractions that are emitted to the water recipient.

Table 3.10. Flows of metals in Stockholm: the influx of metals to the STPs, the effluxes via sludge and treated sewage, including stormwater and groundwater (emitted to surface waters, A), fluxes through the Stockholm Stream (B) and calculated fractions of metals emitted compared to total transports in the recipient and compared to total emissions from the metal stock in Stockholm, respectively. (Data from Bergbäck et al., 2001).

			A	B		
Metal flux, t/y	To STP	In sludge	To recipient	From L. Mälar	A/B, %	A/emiss.,%
Chromium	1.1	0.9	0.40	1.0 – 1.4	29 – 40	47
Copper	10	9	2.63	11 – 18	15 – 24	24
Nickel	2.2	0.8	1.53	10 – 11	14 – 15	235
Zinc	18	12	9.12	17 – 22	41 – 54	38

In absolute numbers, the metal (among those presented in Table 3.10) showing the greatest flux from the anthroposphere of Stockholm to the water recipients around the city is zinc, followed by copper and nickel, while the flux of chromium is much less. However, when these fluxes are compared with the large-scale flux of metals from Lake Mälaren to the Baltic Sea, the contribution from the city of Stockholm is 15-25% for copper and nickel, 30-40% for chromium and 40-55% for zinc. If, on the other hand, the metal discharges to the water recipient are compared with the total emissions from the metal stock in Stockholm, the input to the aquatic ecosystem represents 24% for copper, 38% for zinc, while the figure is higher for chromium. For nickel, those comparisons are not relevant, because the nickel emissions from the metal stock are very low compared to the influx via atmospheric deposition and food and, moreover, the direct input of nickel to the STPs tends to invalidate the comparison.

Table 3.11. Contribution of metals from different sources (%) to the STP of Henriksdal in 1999. After Sörme and Lagerkvist, 2002.

Major route	Source	Cu	Cr	Ni	Zn
Sewage	Households	59	2	16	30
	Businesses	30	13	13	27
	Drainage	2	2	10	4
Stormwater	Buildings	13-17	-	-	24
	Traffic	5	<1	<1	10-11
	Atm. deposit.	<1	1	1	2
Added in STP	Chemicals	-	5	31	2
Total, all sources, %		109-113	23	71	99-100
Total load, kg/y		5560	478	1000	10290

In a later paper, Sörme and Lagerkvist (2002) made a detailed inventory of all the various sources of trace metals that were transported to the biggest STP in Stockholm, Henriksdal. Compared to the previously reported data (e.g. Table 3.10), the percentage of metals entering the Henriksdal STP in 1999, out of the total metal influx to all STPs in Stockholm was, for copper, 56%, for chromium, 43%, for nickel, 45% and for zinc 57%. By making independent estimates of the contribution by every significant source to the mass flow of each of the metals, it was possible to get a general picture of how well relevant sources of metal fluxes were tracked down. The overall results of the estimates for the 4 actual metals are shown, first, in percent of the total amount of metals entering the Henriksdal

STP (Table 3.11), and secondly, in kg/year for the various sources (Table 3.12).

If, based on the figures in Tables 3.11 and 3.12, we compare the rather well-known amounts of incoming metals to the Henriksdal STP, for the year 1999, with the sum of the individual fluxes from all the identified sources, we can find that there is an excellent correspondence in the case of zinc. This was interpreted as practically all the relevant sources of zinc emissions in the reception area of Henriksdal were identified and quantified (Sörme and Lagerkvist, 2002). In the case of chromium and nickel, the correspondence was poor, with only 23% and 71%, respectively, of the incoming metal (to the STP) being accounted for. In the case of copper, it is interesting to notice that the sum of the individual fluxes is greater than the measured total influx to the STP, i.e. some 109-113% of the incoming copper was explained.

Table 3.12. Average emissions in the year 1999 (kg/year) of the four metals, copper, chromium, nickel and zinc, from various sources in Stockholm, within the catchment of the STP of Henriksdal. Estimated according to methods described by Sörme and Lagerkvist, 2002.

Major source	Origin	Cu	Cr	Ni	Zn
Households	Food	275	7	19	2507
	Drinking-water	140	5	141	93
	Water pipes, etc.	2885	-	-	500
Businesses	Large enterprises	87	17	32	200
	Car washes	300	42	39	2300
	Drinking-water	59	2	59	39
	Water pipes, etc.	1200	-	-	200
Traffic	Brake linings	280	0.4	0.4	64
	Tyres, asphalt, oil	15	4	0.6	1020
Infrastructure	Buildings, roofs, etc.	700-920	-	-	2450
Other sources	Drainage, atmos.,chem.	160	38	420	820
Sum of individual contributions		6100-6320	115	710	10190

This phenomenon with a surplus of copper emissions would merit a brief analysis. First, it can be pertinent to compare some of the emission figures in Table 3.12 with emissions estimated in earlier published reports. Looking at the greatest contribution, releases from tap water pipes, heat exchangers and other plumbing materials, originating from both the housing and business sectors, together 4,085 kg/year, it can be noted that this figure is clearly higher than a similar estimate of copper corrosion in the Henriksdal catchment, made for the year 1995, which came to 3,770 kg/year (Landner et al., 2000). The new figure for 1999 is also in poor correspondence with the figure given by Bergbäck et al. (2001) for the whole of Stockholm, 4,300 kg/year. Because Henriksdal deserves about 67% of the total population in Stockholm, and assuming that copper plumbing materials are approximately evenly distributed over the whole urban area, the fraction of the total copper emission from water pipes going to Henriksdal would be about 2,890 kg/year, and not 4,085 kg/y as estimated by Sörme and Lagerkvist (2002).

The traffic sector contributes with a surprisingly low amount of copper in the account made by Sörme and Lagerkvist (2002), i.e. with less than 300 kg/year (Table 3.12). This may seem to be a unreasonably low figure, considering that Bergbäck et al. (2001) reported the total copper emission from traffic in Stockholm to be about 4,100 kg/year. However, Sörme and Lagerkvist start from an estimate of about 1,440 kg/year for the emission from the traffic in the reception area of Henriksdal, where the stormwater collection system is combined with the household sewage collectors. This figure corresponds to the estimate made by Landner et al. (2000) for 1995. Thereafter the authors set out for a reasoning that is somewhat difficult to follow: They start by noting that *"The roads which have stormwater connected to Henriksdal (combined system) are located mostly in the central parts of the city, where most of the surfaces surrounding the roads are hard surfaces."* Meaning that deposited particles from the traffic system to a large degree are transported to the stormwater system and eventually to the STP. In spite of this consideration, they later assume that only about 65% of the brake pad mass loss could be deposited to stormwater (the remaining part obviously remaining as airborne particulate matter). To be sure, Sörme and Lagerkvist, do not even use the latter fraction in their final calculation, but according to a second assumption, they state that only 20% of the total brake lining emission will end up in the STP. Therefore, the traffic contribution to the copper transport to Henriksdal would be only 5% (295 kg/year) of the total.

When Sörme and Lagerkvist (2002) discuss the contribution from copper roofs, however, they use quite a contrasting assumption, namely that there is no retention whatsoever or no losses to other compartments. All the copper released from the roofs situated within the Henriksdal reception area, where there are combined sewer systems (65-85% of the total sewer system area), i.e. 700-920 kg/year, would end up in the STP, according to the authors. They even point out that they have found no scientific evidence of any retention of copper in e.g. sewers made of concrete. Obviously, the authors are unaware of the experimental work carried out by Bertling and others at the Royal Institute of Technology in Stockholm, who have shown that the retention of copper occurring in fresh runoff from copper-clad roofs in soil or concrete varied from 18 to 99.8% (typically in the range 80-90% of Cu retention) (Bertling et al., 2002b). This indicates that runoff water passing a street gutter and/or a collector made of concrete will be significantly impoverished in both free cupric ions and in total copper. It is therefore very abstruse why Sörme and Lagerkvist (2002) have chosen to postulate that 100% of the copper emitted with runoff water from roofs will reach the STP, while only 20% of the copper emitted by traffic in urban streets will have the same fate.

As a matter of fact, the correspondence between total influx to the STP and the sum of the various emissions, that seemed to be very good for copper and zinc at first sight, might not in reality be that good at all, because the conformity with several of the figures presented by other researchers participating in the project “Metals in the Urban and Forest Environment” is poor. However, if a more realistic (higher) figure for the contribution from traffic to the copper transport to Henriksdal is given, and necessary revision of the contributions from water pipes and roofs are made, it is quite possible that the individual fluxes will sum up to close to 100% for copper, as was the case for zinc.

3.3.6 Constraints in metal cycling to arable land with sewage sludge

The EC Directive on sewage sludge (86/278/EEC Directive) provides regulations as to the safe utilization of sewage sludge in agriculture. However, fears among farmers and consumer groups focusing on possible adverse effects of metals, organic contaminants and pathogenic microorganisms in the sludges on soils and crops have resulted in widespread boycotts of sludge in several member states. These aspects are further discussed by Landner et al. (2000) and in sections 4.2 and 5.5.7.

3.3.7 Metal fluxes with groundwater in Stockholm

In the present context, it is also of interest to examine the metal fluxes with groundwater to the water recipients in Stockholm. As part of the Swedish project “Metals in the Urban and Forest Environment”, Aastrup and Thunholm (2001) carried out a great number of measurements of groundwater hydrology and quality. The objectives of this investigation were:

- to get a notion of the concentrations of certain metals in the groundwater of Stockholm; and
- to estimate the metal fluxes with groundwater, in order to reveal the importance of this route, compared with others, for the input of metals to Lake Mälaren and the Baltic Sea.

Sampling of metals was carried out at 70 sites and based on this and other data, a mathematical simulation of groundwater flow and metal transport was conducted to obtain estimates of the total amount of metals stored in the Stockholm groundwater, the total metal transport and the annual leakage of metals into Lake Mälaren and the Baltic Sea. The main results of the study are summarized in Table 3.13 (after Aastrup and Thunholm, 2001).

Table 3.13. Chromium, copper, nickel and zinc in the groundwater of Stockholm. Median and mean concentrations, degrees of elevation above the levels in groundwater in forest ecosystems (nation-wide), total amounts stored in the groundwater, estimates of total fluxes and direct contributions to Lake Mälaren and the Baltic Sea, the latter expressed both as percent of total transport from Lake Mälaren to the Baltic Sea and as percent of the total discharge to water from the city of Stockholm. After Aastrup and Thunholm, 2001.

Parameter	Cr	Cu	Ni	Zn
Median concentration, µg/l	0.79	8.63	7.03	30.7
Ratio median(sto)/median(forest)	3.6	10.3	3.2	1.8
Mean concentrations, µg/l	1.06	12.6	9.59	56.1
Ratio mean(sto)/mean(forest)	2.1	3.3	1.5	1.6
Total storage in groundwater, kg	67	720	590	2570
Total flux with groundwater, kg/y	11	115	94	410
Outflow to L. Mälar + Balt.Sea, kg/y	3.1	34	28	120
% of total flux from L. Mälaren	0.3	0.3	0.3	0.7
% of flux from anthroposphere	0.8	1.3	1.8	1.3

The results of the groundwater studies show that the enhancement of the median or mean concentrations of zinc in the Stockholm aquifer is small (<2 times) compared to corresponding values in groundwaters from forest ecosystems. Also with regard to chromium and nickel, the enhancements of median or mean concentrations are small (<4 times). As far as copper is concerned, the median value from Stockholm, 8.6 µg/l, is ten times higher than the nation-wide forest ecosystem median, but the mean value (12.6

µg/l) is only 3.3 times higher than the “natural” level in forested regions. It should be noted, however, that the bedrock and pre-industrial lake sediments in the Mälaren region hold about twice as much copper as sediments in the great majority of small forest lakes in the country (L & L – Cu). Therefore, the natural background for copper in Stockholm’s groundwater is most probably also twice as high as in the forest ecosystems used for comparison in the table. Thus, the “real” enhancement of the copper median value in Stockholm’s groundwater due to man’s activities might be set at about 5 times.

The total storage of metals in the aquifer was estimated at about 2.6 tonnes for zinc and about a quarter of that quantity for copper (0.7 tonnes) and nickel (0.6 tonnes), while less than 70 kg of chromium were stored in the groundwater.

The direct outflow of metals to Lake Mälaren, the Baltic Sea, including the Brunnsviken Bay, with groundwater is merely a fraction (about 30%) of the amount totally transported with groundwater. Before it reaches the great bodies of water, it flows into streams and small lakes or seeps into tunnels (Aastrup and Thunholm, 2001). It is also clearly shown in Table 3.13 that the fraction of the total flux of metals from Lake Mälaren to the Baltic Sea that originates from the groundwater is extremely small, 0.3% for chromium, copper and nickel, and 0.7% for zinc.

If the groundwater flux of metals is regarded as caused by man, it can be noted that of the total flux of metals from the anthroposphere in Stockholm to the biosphere, the fraction transported with groundwater constitutes 0.8 – 1.8%. As pointed out by Aastrup and Thunholm (2001), the fact that a great part of the precipitation (thus, the runoff) is diverted by land sealing, drainage systems, tunnels, etc. in urban areas, groundwater recharge is usually lower than in rural areas.

3.3.8 Metal accumulation and metal pools in urban soils in Stockholm

As a part of the multifacetted project “Metals in the Urban and Forest Environment”, a special study was conducted with the aim of quantifying the concentrations and pools of metals in urban soils of Stockholm (Linde et al., 2001). In addition to determing the metal concentrations in soils in different parts of the municipality, these concentrations were related to the distance from the city centre and to various types of land use. The amounts of metals per unit surface area were calculated as well as the total accumulated metal pool in the soils of the central city and this was compared with the estimated recent annual input.

The soil types were classified as “city centre soils” (public parks with lawns etc. in the city centre), “undisturbed soils” (areas with semi-natural vegetation), “public park soils” (green areas, excluding those with semi-natural vegetation), “waste land” (mainly former industrial areas), and “roadside soils” (situated within a few metres from the roadway). It is understood that all soil samples except those from soils classified as “city centre soils” were taken from areas outside the immediate city centre. Altogether samples (5-10 from each site) were taken from 42 sites, and data are presented for the surface layer (0-5 cm) and for subsurface soil (30 cm depth); however, data from the subsurface sampling were reported only for 40 sites. A number of soil characteristics were determined and metals were analysed by means of AAS after sample digestion in boiling 7 M HNO_3, according to Swedish Standard (see further details in Linde et al., 2001).

The authors have emphasized that “In order to quantify heavy metal accumulation in urban soils it is of vital importance to establish the background levels originating from the parent material. Rural (arable) soils in the region as well as park soils outside the city centre were found to be useful.” (as reference material). Data on arable soils from the Stockholm region were derived from reports on the metal content in the plough layer (0-20 cm) of 226 samples given by Eriksson et al., (1997; 1999). Concentrations of chromium, copper, nickel and zinc in the different soil types in Stockholm as well as the arable soils in the surroundings of Stockholm are pesented as mean values in Table 3.14.

A certain number of interesting conclusions can be made on the basis of these results:

1. Concentrations of chromium and nickel were low and uniform in all soil types, i.e. there was no tendency to enhanced concentrations of these metals in the city centre soils, when compared to the arable or undisturbed soils.
2. Mean concentrations of copper and zinc were enhanced about 2-3 times in the city centre soils, in comparison with the reference levels in regional arable soils and undisturbed soils within the city.
3. No clear enhancement of the mean copper levels were found in the roadside soils, compared wto the reference material, while zinc levels were about twice as high as zinc concentrations in reference soils. Thus, road traffic, although being an important source of diffuse emission of copper today, obviously has not yet caused a measurable increase in the

5-cm thick layer of soil along the roads, perhaps due to the relatively short time period when copper-lined brakes have been in use.

4. The only soils types showing considerably increased concentrations of copper and zinc were those characterized by former industrial activities (waste land soils), where the copper levels were 10-15 times higher and the zinc levels about 5 times higher than the reference levels. Thus, these results indicate that copper contamination of urban soils appears to be more directly linked to pure industrial activities in the past, and not primarily a result of a general diffuse release from various goods in use.

Table 3.14. Concentrations of chromium, copper, nickel and zinc (as mean values of total metal in mg/kg dry weight) in various soil types in the city of Stockholm and in arable soils in the Stockholm region. After Linde et al., 2001 and Eriksson et al., 1997 and 1999.

Type of soil	Depth	Cr	Cu	Ni	Zn
City centre soils	0-5 cm	27	47	9.0	157
	30 cm	27	67	10.6	193
Undisturbed soils	0-5 cm	49	23	14	76
	30 cm	35	23	16	80
Park soils	0-5 cm	35	30	17	140
	30 cm	40	30	22	100
Waste land soils	0-5 cm	44	290	15	400
	30 cm	36	140	18	130
Roadside soils	0-5 cm	25	27	11	130
	30 cm	36	28	19	150
Arable soils	0-20 cm	29	19	15	72

Linde et al. (2001) have also calculated the average pools of metals in the various soil types in Stockholm. Calculations were made for the upper 5 cm of city centre soils, park soils and arable soils, and for the upper 30 cm of city centre and park soils. Moreover, the total pools of metals in city centre soils were computed (based on a total area of 4.5 million m^2) together with the difference in total pools between city centre soils and park soils, in order to provide a rough estimate of the amount of metals accumulated in the city centre soils due to local urban activities. The results for the 0-30 cm depth layer are presented in Table 3.15. The table does not include data for chromium and nickel, because the pools of these metals were smaller in the city centre soils than in park soils and in arable soils. In contrast, data on lead are included for the sake of comparison.

Table 3.15. Calculated average pools of copper, lead and zinc (g/m²) in the top 30 cm of soils in the city centre of Stockholm as well as in parks outside the city centre. Data are also given on total pools, together with estimates of amounts of metals accumulated due to local emissions (given in tonnes). After Linde et al., 2001.

	Average pools (g/m²)		Metal pools in city centre soils (tonnes)	
Metal	Park soils	City centre soils	From local emissions	Total
Copper	12	21	40	93
Lead	12	38	120	172
Zinc	40	58	83	260

The average pools in city centre soils (0-30 cm) of lead was 3-4 times higher and of copper and zinc about 1.5 – 2 times higher than the background level.

The authors (Linde et al., 2001) also concluded that the estimates of annual emissions of copper and zinc to the Stockholm soil environment, 4 tonnes and 15 tonnes, respectively, made by Sörme et al. (2001b) and Bergbäck (1998), seem to be rather unlikely. This is because the accumulated metal amounts in the city centre would be the result of only 10 years of emissions in the case of copper and about 6 years of emissions in the case of zinc. The explanation of this difference might be either that only small fractions of the copper and zinc released from the technosphere end up in the city centre soils or that the total diffuse emissions in reality are smaller than those estimated by Sörme et al. (2001b) and by Bergbäck (1998).

As general conclusions, Linde et al. (2001) noted that their study showed:

- that more homogeneous soil groups – with regard to metal contamination – were obtained based on present land use than on geographic distance to the city centre;
- the importance of establishing a background level of metal concentrations in order to quantify the degree of local contamination; and
- that soil samples have to be taken below the surface layer (and deeper than 30 cm) in order to quantify the accumulated metal pools in urban soils.

3.3.9 Metal fluxes to sediments of lakes and coastal areas in Stockholm

The possibility of using bottom sediments in lakes and inlets of the Baltic Sea to quantify the annual loads of metals from the Stockholm area, in particular metal fluxes resulting from diffuse urban emissions, was investigated by Lindström (2001) and by Lindström et al. (2001).

In the study forming part of the collective project "Metals in the Urban and Forest Environment" (Lindström et al., 2001), the Stockholm archipelago area and the eastern-most parts of Lake Mälaren were divided into 14 subareas, mainly along a transect corresponding to the principal flow of water, to investigate the metal load on the sediments. During two sampling cruises in August 1997 and June 1988, 5-7 sediment samples from accumulation bottoms in each of the defined sub-areas were collected. Two or three cores per sub-area were dated using the ^{137}Cs technique.

Sediment samples (<0.5 g) for metal analyses were digested in 20 ml of 7 M HNO_3 at 120 °C for 30 min. (according to Swedish Standard), and then analyzed using ICP-MS. Average annual deposition of metals in each sub-area was determined by multiplying surface sediment (0-2 cm) metal concentrations (µg/g dry sediment, ds) with mean sediment deposition rates (g ds/m^2, y) from the dated cores and areas of accumulation bottoms (m^2).

The estimated total metal deposition in the combined investigated sub-areas situated in Lake Mälaren close to the city of Stockholm as well as in the combined sub-areas in the archipelago (Baltic Sea) close to the city are presented in Table 3.16, together with the estimated range of current metal sediment load from Stockholm and the average metal fluxes with water flowing from Lake Mälaren to the sea. All fluxes are given in t/y, after Lindström et al. (2001).

In order to estimate the metal load on the sediments from the city of Stockholm, the deposition had to be corrected for the background deposition. The approach chosen to estimate the background deposition was to utilize the metal concentrations from the outermost sampling points as reference values, and then correct these concentrations to account for processes influencing the natural metal concentration (reduction/oxidation reactions and desorption). Therefore, the reference values were normalised to an element that has only (or almost only) natural sources and similar sedimentological properties as the elements of interest, such as Sc, Al or Ti. It turned out that also Ni could be used for normalizing purposes, since it was not possible to detect any enrichment of Ni in central Stockholm compared to Sc. The ranges of the Stockhom metal load on the sediment were then calculated from the 95% confidence intervals of the ratios of Cr, Cu and Zn to Ni (Lindström et al., 2001).

Table 3.16. Estimates of total metal deposition to sediments in Lake Mälaren and in the Baltic Sea archipelago close to the city of Stockholm, and of the sediment metal loads originating from Stockholm (max and min), as well as the average metal flux from Lake Mälaren to the Baltic Sea in the period 1995-1997. All fluxes in t/y. After Lindström et al., 2001.

Type of flux	Areas	Cr	Cu	Ni	Zn
Total deposition to sed.	L.Mälaren	1.13	1.54	1.18	5.91
	Archipel.	3.55	5.56	2.15	16.8
	Combined	4.7	7.1	3.3	23
Load to sed. from Sthlm	Min	0.3	1.5	--	3.6
	Max	1.2	4.5	--	12
Flux with outflow from L.Mälaren		1.2	14	11	21

If the metal amounts deposited in the innermost archipelago exceed the outflow from Lake Mälaren (Cr is an example of this), there must exist other sources of metals than the surface water outflow to explain the deposition. The authors discuss such potential sources, such as metal fluxes with ground water and with effluents from sewage treatment plants, but conclude that these are very small to small compared to the outflow from Lake Mälaren (Lindström et al., 2001). Also the direct atmospheric deposition to the water surface appears to be low (<20% of the metal fluxes with the Lake Mälaren outflow, according to the analytical laboratory, SLB, 1998).

The possible explanation to the above phenomenon, put forward by Lindström et al. (2001), is that there might be a large inflow of Baltic Sea water into the Stockholm archipelago. To support this hypothesis, they mention the observed metal depositon pattern and the surprisingly high ^{137}Cs inventories (10-40 kBq/m^2) in these archipelago areas, in spite of the low direct fallout (0-2 kBq/m^2), as pointed out by Meili et al. (2002).

As can be seen in Table 3.16, the estimates of the metal loads to the sediments originating from the city of Stockholm show a great uncertainty, with a range of 3-4 times between the minimun and maximum value. There may be a different approach to determining the contribution from Stockholm city to the metal burden of the sediments. This approach is based on the rather simplistic assumption that the increase in relative metal deposition between Lake Mälaren and the innermost Baltic Sea archipelago would very roughly reflect the contribution from the city of Stockholm. However, the total metal deposition values for the two areas, uptream and downstream of

Stockholm, given in Table 3.16 have to be corrected for difference in total surface area and difference in the average percentage of accumulation bottoms in the two regions, acccording to data from Lindström et al. (2001). Normalisation of the metal deposition data has been made as follows:

The combined surface area of the innermost archipelago, used to calculate total metal deposition is 123.5 km², i.e. 1.49 times larger than the corresponding surface area in Lake Mälaren, 83 km². The average percentage of accumulation bottoms is 1.14 times greater in the archipelago region than in the Lake Mälaren regions under investigation. The product of these relations is 1.7.

- **Cr**: 3.55 / 1.7 = 2.09 (normalised deposition in innermost archipelago); thus, the approximate contribution from Stockholm would be: 2.09 – 1.13 = 0.96 t/y;
- **Cu**: 5.56 / 1.7 = 3.27 ; thus the contribution from Stockholm would be about 3.27 – 1.54 = 1.73 t/y;
- **Ni**: 2.15 / 1.7 = 1.26; thus Stockholm would give: 1.26 – 1.18 = 0.08, i.e. close to zero contribution;
- **Zn**: 16.8 / 1.7 = 9.88; thus Stockholm would contribute 9.88 – 5.91 = 3.97 t/y.

Using this simplified method to roughly estimate the contribution from Stockholm to the total deposition of metals to the sediments in the innermost archipelago of the Baltic Sea, we arrive at almost the same conclusions as did Lindström et al. (2001). The city of Stockholm seems to be insignificant for the sediment loading with nickel, which is in accordance with the view expressed by Lindström et al. (2001). The contribution to the chromium load to sediments (about 1 t/y) is close to the maximum value calculated earlier, while the now calculated loads of copper and zinc to sediments (1.7 and 4.0 t/y, respectively) are close to the minimum levels calculated by Lindström et al. (2001). Of the total amount of metals deposited in the sediments in the innermost archipelago, it can be estimated that about 30% of the chromium and copper originated from Stockholm, while less than 25% of the zinc had the same origin. The contribution of the city to the nickel deposition in the sediments was almost nil.

If we compare the above calculated contributions from Stockholm to the metal loads on sediments in the innermost archipelago with the combined measured metal fluxes from STPs, with storm water and ground water (i.e. the waterborne fluxes from the anthroposphere), see Table 3.10, the following picture emerges:

- **Cr**: 0.4 t/y (measured efflux) to be compared with 1.0 t/y (from Stockholm to sediment) – additional sources existing, to explain higher depositon load;
- **Cu**: 2.63 t/y (measured efflux) to be compared with 1.7 t/y being deposited – a relatively small net amount of the city's emissions seems to be transported further out to the sea;
- **Ni**: 1.53 t/y (measured efflux) to be compared with 0.08 t/y being deposited – indicating a considerable amount being transported towards the sea;
- **Zn**: 9.12 t/y (measued efflux) to be compared with 4.0 t/y being deposited – a relatively large net amount appears to be transported towards the sea.

Finally, the relation between the total metal deposition on sediments in the innermost archipelago and the total metal flux through the Stockholm Stream shows that the most mobile element of the four being in focus here is nickel (only 20% of the flux is deposited), copper has an intermediate mobility (40% deposited), and zinc is the least mobile of the four elements with 80% of the flux being deposited. For chromium, the deposition is greater than the flux through the Stockholm Stream. The indicated relative mobilities of the metals are not in accordance with conventional knowledge, according to which zinc usually is considered a mobile element. These unexpected relationships emphasize the risks involved in using relatively uncertain data for far-reaching conclusions, and brings attention to the fact that the establishment of mass balances of environmental elements based on sedimentological studies is very difficult and calls for a thorough scrutiny of methods used. Just to mention one limitation in the possibility to use Lindström's et al. (2001) work for calculating the relative contribution of the city of Stockholm to the total metal loads to sediments, it should be mentioned that some of the investigated sub-areas in the archipelago (e.g. sub-areas 3, 4 and 5) are influenced not only by the emissions from the city of Stockholm, but also by STP discharges (e.g. the Käppala STP), treating sewage from other towns. Therefore, the amount of metals in Baltic Sea sediments that were estimated to originate from the city is, in reality, an overestimate of the contribution from Stockholm.

Nonetheless, Lindström et al. (2001) have provided a much more reliable picture of the real impact of the infrastructure and activities in Stockholm City on sediment ecosystems in the surrounding waters than has been given by some other authors in their recent reports. Thus, unfortunately, certain reports, from studies of metals and organic contaminants in sediments of waters surrounding Stockholm (e.g. Östlund et al., 1998; Sternbeck and Östlund, 2001; Broman et al., 2001), have presented rather misleading conclusions on the actual role of the city in explaining the total load on nearby sediments. However, in a later raport by Sternbeck et al. (2003), some of the previous overestimates have been rectified (cf. section 4.5).

In none of the above-mentioned studies from 1998-2002, there was any serious attempt to estimate the natural background concentration of metals in the region, which makes it almost impossible to assess the relative contribution from the city. Moreover, in the latter of the three earlier studies (Broman et al., 2001), the deposition of contaminants to sediments in the innermost Baltic Sea archipelago was overestimated about three times due to a calculation error.

Because of these shortcomings, an approach is proposed in this report to how the regional, natural background concentrations of trace metals in aquatic sediments may be established. This proposal is included as section 5.7, after a thorough discussion about speciation and bioavailability of trace metals in the environment.

4 CRITICAL STEPS IN METAL FLUXES FROM SOCIETY TO THE ENVIRONMENT – SOME CASE STUDIES

In the research conducted over the past few years with the double aim of closing cycles of trace metals in society and understanding the relative importance of different sources and fluxes from the anthroposphere to the environment, a certain number of “key questions” or “critical steps” have been identified. It was found that more comprehensive investigations were needed to further elaborate on these problems, the resolution of which would considerably improve our overall understanding of the processes involved. Among these “key questions”, which have been the focus of considerable research efforts and – consequently – on which substantial new information has been produced since the compilation of the three original monographs on Metals in Society and in the Environment, the following ones appear to be particularly important and will be treated in this chapter:

- the flux of copper, zinc, chromium and nickel from buildings and constructions in the urban environment as a result of corrosion and runoff;
- the flux of trace metals from the traffic sector in built-up city areas to the aquatic environment, and to aquatic organisms in the near-field recipients;
- the flux of trace metals from households, including the plumbing systems in buildings, to sewage treatment plants (STPs), sewage sludge and agricultural soils;
- the flux of copper and zinc from mine wastes and long-term contaminated soils to surface waters (lakes, rivers, the sea), as exemplified by the Falu Copper Mine;
- the flux of metals from major point sources of metal emissions through the atmosphere and soils in the surroundings of the emission sources.

4.1 Case Study 1: Corrosion and runoff of metals from roofing materials made of copper, galvanized steel or stainless steel (Cu, Zn, Cr and Ni)

A team of researchers at the Division of Corrosion Science, KTH, conducted systematic studies of corrosion and metal runoff from sheets (with different age) made of copper, galvanized steel or stainless steel, exposed on roofs in the urban atmosphere in Stockholm. The main aim was to determine the rates of both corrosion and metal runoff under various environmental conditions. Also the speciation and fate of released metals (Cu and Zn) were studied during their transport from the roofs to the natural receiving bodies of water.

The atmospheric corrosion rate for both Cu and Zn typically changes with time. In contrast, the rate of metal runoff from the surfaces is much more constant in time. These basic findings were shown to be valid under different atmospheric conditions (air qualities, rain pH, etc.). In the initial phase of panel exposure, the runoff rate of both metals was lower than the corrosion rate, leading to continuous accumulation of metal in the corrosion products layer, remaining on the panel. After some time, the runoff rate will equal the corrosion rate, which happens on Zn after a few years exposure in Stockholm and probably after a few decades for Cu. For Cu sheet aged from 0 to 130 years, the runoff rate was confirmed to be in the range 1.0 – 2.0 g Cu/m^2*, y, and the Zn runoff rate was found to be about 3.1 g Zn/m*^{2}*, y. Both rates are specific for the Stockholm urban atmosphere with low concentrations of sulphur dioxide (about 3 µg/m*^{3}*) and annual rainfall of about 540 mm. The corresponding release rates of Cr and Ni from stainless steel were found to be 0.2 – 0.7 mg/m*^{2}* and 0.1 – 0.8 mg/m*^{2}*, respectively, i.e. some 10,000 times lower than for Cu and Zn.*

By using sophisticated metal speciation approaches, the Cu and Zn species in the runoff water were described. At the edge of the investigated roof, 60-100% of the released Cu was present as free hydrated cupric ions, while 95-99.9% of all Zn in the runoff was present as free, hydrated Zn(II) ions. Depending on the pH and sulphate content, $Zn(OH)^+$ *and* $ZnSO_4(aq)$ *could also be present. Measurements with Cu- or Zn-specific biosensors and tests for growth inhibition with green algae confirmed that almost the total amount of the metals occurred in a bioavailable form in the runoff just at the edge of the roof. However, in tests where the Cu- or Zn-containing runoff* *percolated through a soil column, the retention of total Cu and Zn was found to be 96-99.8% and the fraction of bioavailable metal in the percolate was significantly reduced. Other tests with Cu-containing runoff in contact with concrete or limestone indicated that also these materials partly retained the metal.*

It was concluded that Cu and Zn, occurring in the runoff from roofing materials and other structures exposed in the urban atmosphere, are initially highly bioavailable, but during transport from the site of origin towards the final recipient, the runoff will be in close contact with soil and other reactants that considerably reduce the amount of bioavailable metals. It is pointed out that the bioavailability of metals has a real meaning only in the receiving environment.

4.1.1 Definitions, background and experimental approaches

Metals and metal containing products are used in large amounts in buildings and in constructions making up the infrastructure of modern society. Among the frequently used metals in building applications, such as building shells, roofing materials and fencing, are copper (in pure form or as a copper-based alloy), zinc (mostly as a plating on steel), and chromium and nickel (as alloying elements of stainless steel). Their dispersion is caused by atmospheric corrosion and the subsequent precipitation-induced release (the “runoff”) process (Odnevall Wallinder and Leygraf, 2002).

Before looking closer at these processes, it may be pertinent to define some of the key concepts in this field:

Corrosion rate is the amount of a metal (g) that corrodes per surface area unit (m^2) and time unit (year). The rate is given in $g/m^2/y$. Part of the corroded metal will precipitate and remain on the metal surface as a corrosion product, whereas another part will run off during precipitation events. The *runoff rate* is the amount of metal (g) that is released from the corrosion product per surface area unit (m^2) and time unit (y).

Corrosion and runoff are generally governed by completely different physical, chemical and electrochemical processes. It follows that corrosion and runoff proceed independently of each other and with rates that usually are not equal (Odnevall Wallinder and Leygraf, 1997). Differences between corrosion rate and runoff rate can mainly be attributed to successive accumulation – or reduction – of metal in the corrosion product. The metal released with the runoff occurs in a chemical form (as a chemical species) with specific reactivity, mobility and bioavailability, depending on the ambient chemical and physical environment. In order to determine the environmental hazard of metals released as a result of corrosion and runoff from metal coatings and metallic materials used on buildings and constructions, it is obviously of great importance to carefully examine the properties (speciation) and the fate of the metal during its successive transport away from the original site of release. Such a holistic approach was chosen by the team of researchers at the Division of Corroson Science, KTH, Stockholm, under the leadership of C. Leygraf and I. Odnevall Wallinder (op. cit., 2002).

Although the rates of corrosion and degradation of different materials have been studied extensively for many years, systematic investigations of runoff from metallic surfaces have been rare. The first scientifically well-planned runoff studies in Sweden were implemented by the KTH team in 1995, and have ever since been continued to cover all relevant materials and various aspects of the problem (Odnevall Wallinder and Leygraf, 2002). As a parallel effort, studies of metal runoff from about a dozen different common roofing and facing materials (including various paints) were conducted in 1997-98 after both short-term and long-term (up to 1 year) outdoor exposures in the Stockholm atmosphere by the Swedish Corroson Institute (Persson and Kucera, 2001). Thus, a considerable amount of data has been collected over the past few years on the corrosion – and particularly on the runoff – process, together with detailed examination of the fate, bioavailability and ecotoxicity of the released metals. In fact, several academic theses have already resulted from this work (He, 2000; 2002; Karlén, 2001).

The experimental approaches to measuring the rate of metal runoff from various materials has been a combination of field experiments and laboratory follow-up studies. During the many individual studies that were conducted as complements with the aim of broadening the basic knowledge obtained during long-term (up to 4-5 years) continuous exposures of materials to well-defined atmospheric conditions, several specific questions have been addressed:

- How does the air quality (urban – rural air) influence metal runoff ?
- How does temperature, rain intensity and length of dry periods affect metal runoff ?
- How does metal runoff change during a rain episode (effect of first flush) ?
- What is the role of pH of precipitation ?
- How does surface inclination and wind exposure, etc. affect metal runoff?

- What is the effect of age of the roofing material (natural or artificial patination) ?
- How large quantities of metals are dispersed via corrosion and runoff from buildings ?
- Can artificial rain in the laboratory be used to simulate natural rain ?
- What chemical species of metals occur in the runoff water leaving the roof ?
- How does the chemical speciation of metals change when the runoff reaches the ground?
- What are the bioavailability and toxicity properties of metals in runoff, immediately and after transport in contact with limestone, concrete and/or soil ?

4.1.2 Some principal results

Before going into a more detailed description of the results obtained from exposures of individual metal plates (copper sheet, galvanized steel, stainless steel), a few key observations from the runoff studies will be presented. It is well-known that the corrosion rate for both copper and zinc changes quite conspicuoulsy with time, showing an initial high rate that successively levels off. In contrast, the runoff rate is much more constant in time, at least seen at time scales of one to several years (Odnevall Wallinder and Leygraf, 2002). Since this behaviour has been repeatedly observed with both copper and zinc, in several different atmospheric environments, and in spite of the significant changes in chemical composition the corrosion products undergo with time, it was concluded that the runoff rate of copper and zinc is basically governed by precipitation parameters, including air quality and rain pH.

In the beginning of the exposure to the atmosphere, the runoff rate of both metals is lower than the corresponding corrosion rate, leading to a continuous accumulation of metal in the corrosion products, remaining on the panel. Eventually, the runoff rate will equal the corrosion rate, which is reflected by the discontinuation of the growth in mass of corrosion products. This happens on zinc after about a few years exposure in the Stockholm atmosphere, and probably after a few decades for copper (Odnevall Wallinder and Leygraf, 2002). The age of corrosion products on copper and zinc does not seem to have any major influence on the runoff rate, at least for up to 140 years old copper sheet (Odnevall Wallinder et al., 2002a) and 40 years old zinc sheet (He et al., 2002).

4.1.3 Releases and fluxes of copper

Corrosion of metals forms part of the natural cycle in which the metal is striving through spontaneous chemical and electrochemical processes to reach back to its most stable condition, i.e. as a mineral, from which it was originally refined. When copper sheet is used as a roofing material, it undergoes gradual degradation, induced by wet and dry deposition of environmental pollutants, such as SO_2, NOx, O_3, HCl and NaCl. A prerequisite for a corrosion process to occur is the presence of water, e.g. humidity, dew, fog or precipitation. He (2002) has made a survey of the most common corrosion products, formed on a copper roof in an urban environment:

The product formed first (within minutes or hours) is cuprite, Cu_2O, which may transform into the intermediary amorphous copper sulphate and then – after months or years – into any of the two (greenish) copper sulphate hydroxides, posnjakite and brochantite (usually known as "patina"). In chloride-containing environments such as road traffic or marine atmospheres, the copper chlorides nantokite (CuCl) and atacamite ($Cu_2Cl(OH)_3$) may form. Typical corrosion rates, expressed in µm/y, have been given as ~ 0.5 in rural environment, ~ 1.0 in marine, 1-2 in urban, and ≤ 2.5 in industrial environment (Leygraf, 1995).

When both corrosion rate and runoff rate on fresh copper sheet are expressed in g/m^2, y , the relationship between the two, when measured over 48 weeks of exposure to the urban atmosphere in Stockholm (annual precipitation 575 mm, average atmospheric concentration of SO_2 = 3 µg/m^3 and of NO_2 = 50 µg/m^3), is as follows: the annual corrosion rate was 6.7 g Cu/m^2, y and the runoff rate 1.3 g Cu/m^2, y (He, 2002). Exposure of a naturally (in situ, in the urban environment) patinated copper sheet of an age of 100 years, resulted in a somewhat higher annual runoff rate: about 2.0 g Cu/m^2, y. In laboratory follow-up experiments it was shown that the "first flush" (rain on a dry surface) contributed to the total runoff with a higher percentage (44%) from the 100-year old copper plate than from the fresh copper plate (18%).

Additional measurements (Karlén et al., 2002; Odnevall Wallinder and Leygraf, 2001) have confirmed that runoff rates of copper from the urban testing site in Stockholm varied between 1.0 and 1.7 g Cu/m^2, y for fresh copper (<5 years old) and between 1.3 and 2.0 g Cu/m^2, y for green, naturally patinated copper (>30 years old). The variation within each category of panel age was mainly attributed to differences in annual precipitation quantity. The reason for the discrepancy in runoff rates between fresh and old copper plates (primarily during the first flush) was due to the morphology and other surface characteristics of the corrosion products within the patina. For example, the adsorption capacity of water was higher in the porous structure of green-patinated plates. Hence, corrosion and dissolution can take place even during relatively dry periods, since water is trapped within the patina. Therefore, the first flush effect is more pronounced on green-patinated copper compared to brown-patinated copper (Karlén, 2001; He, 2002).

During extended exposures for more than four years of both fresh copper sheet and naturally patinated, 130 years old copper sheet in the Stockholm atmosphere, further confirmation was obtained that the annual runoff rates were 1.1 – 1.6 g Cu/m^2, y and 1.6 – 2.0 g Cu/m^2, y, respectively (Odnevall Wallinder et al., 2002a).

In order to assess the influence of the amount of precipitation and of the air quality on the runoff rate, a comparative study was set up with simultaneous copper sheet exposures in Singapore and Stockholm. The total annual rainfall is 5-8 times higher in Singapore compared to Stockholm and also the rain frequency and duration of humid periods are much higher. Moreover, the atmospheric concentration of SO_2 is 7-8 times higher (about 20 µg/m^3) in Singapore compared to Stockholm. After about one year of exposure, it was found that runoff rates from fresh copper sheet in Singapore were 5.6-5.7 g Cu/m^2, y. At the same time, the copper runoff from pre-patinated copper sheets was estimated, and it was found that although the Singaporean runoff rate from brown pre-patinated sheet was almost identical with that from fresh copper sheet, the runoff rate from green pre-patinated sheet was significantly higher, 8.4-8.8 g Cu/m^2, y (Odnevall Wallinder et al., 2002a).

Previous calculations carried out in 1998 (L & L – Cu) came to the conclusion that the total amount of copper primarily washed off from copper roofs and copper-faced façades in Stockholm would be in the range 1.1 – 1.3 t/y. Now, with the more refined measurements of the runoff from copper roofs of different ages and with a more sophisticated assessment of the total surface of copper coated buildings in Stockholm, based on digital air photo processing (Ekstrand et al., 2001), it turns out that the new and refined estimates end up with a somewhat smaller figure: on average, 1.0 t of copper leaves the copper coated roofs every year.

It should, however, be clearly recognized that the indicated quantity of copper is what is released from the roofs in the city. In order to assess the further fate of this quantity of copper, it is important to undertake a thorough speciation of the metal, in particular determine the reactivity, mobility and bioavailability of the released metal. This has been the subject of a licentiate thesis, prepared by one member of the KTH team (Karlén, 2001), and of one of the included papers, specifically dealing with copper (Karlén et al., 2002).

Samples were taken at the edge of the roof, equipped with either a fresh copper panel or with a naturally patinated, 30 years old, copper sheet, which were exposed to the Stockholm atmosphere for 3 years. The methods used for speciation of copper were:

- measurements of the cupric ion concentration using a ion selective electrode, ISE (ORION Model 9629);
- computer modeling, using the water-ligand model MINTEQA2 (Allison and Brown, 1991), with input data of pH, alkalinity and concentrations of sulphate and chloride in the runoff water;
- use of the cell-based biosensor BIOMET (a gene-modified strain of *Alcaligenes eutrophus*), specifically developed for detection of bioavailable copper (Corbisier, 1997);
- application of the OECD “Algal Growth Inhibition Test” to detemine the copper concentration in the runoff causing 50% inhibition of algal growth, and then relate this concentration to the total concentration of copper in the runoff water.

Karlén et al. (2002) confirmed the previous, repeatedly found, results that annual runoff rates varied between 1.0 and 1.5 g Cu/m² for naturally patinated copper sheets of varying age. The concentration of total copper in the runoff water samples ranged from 0.9 to 9.7 mg/l. Both computer modeling and direct measurements by means of ISE and the biosensor revealed that nearly all the copper in the runoff water, sampled directly at the edge of the roof was bioavailable. Some 60-100% of the released copper was present as free hydrated cupric ion, $Cu(H_2O)_6^{2+}$, which is considered the most bioavailable copper species. Other copper species in the runoff water, such as $Cu(OH)^-$ and $Cu_2(OH)_2^{2+}$, are also bioavailable.

Testing of toxic effects on the alga *Raphidocelis subcapitata,* during standard exposure for 72 hours, demonstrated that the toxicity of the runoff water leaving the roof was high: the EC_{50} value ranged from 0.29 to 0.67 vol-% (150-350 “Toxic Units”, TU = 100/ EC_{50}). When the 72-h EC_{50} values for the runoff water were expressed in µg of total Cu per litre, it was clearly demonstrated that the range (6-24 µg Cu/l) was very close to the 72-h EC_{50} reference value for $CuSO_4$ (18-27 µg Cu/l), mainly representing free cupric ion. This means that the same chemical species, or species with the same degree of bioavailability, would have been present in the runoff water.

After having provided a satisfactory description of the copper species occurring in the runoff water immediately after leaving the roof, the next step is to evaluate what transformations of these copper species may take place, when the runoff water comes into contact with various sorbing materials and solid surfaces on its way to the STP or to the final receiving body of water. It is assumed that – during further transport in roof gutters, downpipes, street gutters and sewers – copper undergoes changes in chemical speciation, which may result in immobilisation of some of the copper, thus reducing the amount transported further on, and possibly reducing the bioavailability of the remaining amount.

These aspects have been studied by Bertling and co-workers, first in a laboratory study, where the capacity of limestone to retain and immobilize copper was investigated (Bertling et al., 2002a). A series of follow-up field and laboratory investigations were then conducted, where runoff water, containing copper, was passed through reactors containing various sorbing surfaces, such as limestone, soil or concrete, that mimic the normal situation prevailing where runoff water encounters solid materials during environmental entry (Bertling et al., 2002b).

In the laboratory study, which was conducted according to a fractional factorial design, a great number of parameters that may influence the copper immobilization by limestone were tested. The most significant parameters were the specific surface area of limestone, the copper concentration in the runoff water and the amount of limestone used in the reactor (Bertling et al., 2002a).

In the follow-up experiments, the runoff water was allowed to interact for short periods of time with limestone, concrete or soil (natural European soils and an OECD standard soil).

The retention capacity of copper appeared to be highest in soil (96-99.8%), but also quite substantial in concrete (18-95%) and limestone (5-47%). The interaction between the copper-containing runoff water and the interfaces in these materials also caused a significant reduction of the fraction of free, hydrated cupric ion, thereby drastically decreasing the concentration of bioavailable metal (Bertling et al., 2002b).

Thus, roof runoff water containing copper, where the dominant fraction consists of free cupric ions when the runoff is leaving the roof, will rapidly become impoverished in both free cupric ions and total copper, due to the high reactivity of the metal species when interacting with materials that are common in the catchment of the runoff water. So far, it has not been possible to quantify the amount of copper being retained in the close surroundings of copper roofs, e.g. in Stockholm. Nor has it been possible to evaluate the impact of copper roofing and facing materials on the range of concentrations of cupric ions – and other bioavailable copper species – in receiving waters where protection of natural biota has a high priority.

For the time being, it is only possible to make an educated guess – very similar to the speculation made in (L & L – Cu) in 1998 – that of the 1.2 t/y of total copper being released from copper roofs and façades in Stockholm, at least half of the released amount is retained in solid materials close to the source. Thus, about 0.6-0.7 t/y of total copper would be physically transported away from the close surroundings of copper-clad buildings, and of this amount, some 50-60% would still be in a bioavailable form. During further transport in the storm-water sewers – or combined sewers – (often made of concrete), an additional fraction of unknown magnitude of the released copper will be immobilised and retained before the runoff water arrives at the STP.

4.1.4 Releases and flows of zinc

Zinc has a relatively good resistance against corrosion, and the metal has therefore been widely used as a surface protecting agent, e.g. in alloys with aluminium and as a coating for steel (galvanizing) on buildings and infrastructure elements. Nonetheless, corrosion of zinc-containing materials, exposed to urban atmosphere with its various air pollutants, is an important phenomenon, not least because of the potential environmental impact that the released metal could cause (see e.g. IPCS, 2001). Depending on the prevailing environmental conditions, a certain fraction of the corrosion products formed will dissolve and be flushed from the surface of, e.g. a roof during a rain event, while another fraction will re-precipitate to the adhering corrosion product layer (Karlén et al., 2001).

During urban atmospheric exposure, zinc forms relatively porous and voluminous patina layers, which are primarily composed of basic zinc carbonate, $Zn_5(CO_3)_2(OH)_6$, and basic zinc sulphates, $Zn_4SO_4(OH)_6$ x nH_2O ($n = 1$-5) (Odnevall and Leygraf, 1995). In the case of zinc corrosion, much more than in the case of copper corrosion, one atmospheric pollutant, SO_2, is predominant in governing the corrosion attack. It has been repeatedly demonstrated that there is a strong correlation between the atmospheric SO_2 concentration and the zinc corrosion rate. Thus, the substantial reduction in SO_2 concentration in urban atmosphere over the last decades has been followed by a sharp drop in the zinc corrosion rate. However, not only the rate of zinc corrosion, but also the zinc runoff rate has been significantly reduced as a result of declining levels of atmospheric pollutants (Odnevall Wallinder et al., 1998).

It has been attempted to derive empirical relations between concentrations of SO_2 and the rates of corrosion and runoff, based on measurements at exposure sites with largely different SO_2 levels, but with similar annual quantities of precipitation. This was obtained with data from recent measurements up to 4.5 years in rural, urban and industrial atmospheres in Belgium, France and Sweden. The relations, derived by

Odnevall Wallinder et al. (1999), are the following, where SO_2 concentration is given as µg/m³, and the corrosion rate and the runoff rate as g/m², y:

$$\text{Corrosion rate} = 3.78 + 0.23\,(SO_2)$$
$$\text{Runoff rate} = 1.36 + 0.16\,(SO_2)$$

As can be seen from these relationships, the runoff rate is considerably lower than the corrosion rate; runoff rates are 40-65% of the corrosion rates for SO_2 levels <50 µg/m³.

Systematic studies of the runoff rate from zinc-containing materials during atmospheric exposure, where the variation in runoff rate as a function of various environmental conditions has been examined in detail, were carried out by the KTH research team, the results of which have been reported by e.g. He (2000; 2002) and He et al. (2001; 2002). It was shown that readily soluble and poorly adhesive corrosion products were released from the corrosion- product layer during the first flush event. This resulted in an initially high concentration of zinc in the runoff water, followed by a decreased, and after some time, almost constant metal concentration. The magnitude of the first flush and, hence, the quantity of released metal depends on the environmental conditions prior to the rain episode, characterized by dry deposition of corrosive agents and by the length of dry periods, as well as on the quantity and intensity of the following rainfall. The amount of zinc in the runoff water increases with decreasing pH, during the first flush period as well as during the steady state period of a rain episode (He, 2000).

Yearly runoff rates for zinc as compiled by Karlén et al. (2001) ranged between 0.07 and 3.5 g/m², y, based on 1-year exposures of panels in the Stockholm urban atmosphere (SO_2 concentrations about 3 µg/m³ and an annual rain quantity of about 540 mm). The differences in runoff rates are related to the presence of various barriers, such as different commercial coatings (Table 4.1). Such coatings could reduce the amount of zinc in the runoff by a factor between 2 and 50, compared to a non-coated surface. The presence of barriers also resulted in different pH (range: 4.5 – 7) of the collected runoff water, as a result of varying buffering capacity of the dissolved zinc corrosion products. The blank surface (plexiglas) was used to measure the contribution of atmospheric deposition of zinc; this contribution was small, being less than 0.02 g/m², y.

Continued experiments in Stockholm with exposure periods for up to four years have provided long-term results showing that the accumulated zinc runoff quantity increased linearly with time ($R^2 = 0.99$). The slope of the curves corresponded to an average runoff rate of 3.1 g/m², y and 3.2 g/m², y for new and 40 year old zinc sheets, respectively (He et al., 2002).

When the runoff rates from zinc panels of three different ages (unaged, 15 and 39 years old) were compared during field exposure for 48 weeks, it was demonstrated that there was *no significant age effect* in runoff rate, all three types of panels gave about 3.1 g/m² (He et al., 2001).

Table 4.1. Annual zinc runoff rates from five major groups of zinc-containing commercial building materials used in Sweden, as well as individual values for each material, annual rates given in g/m². After Karlén et al., 2001.

Group	Material (specification)	Runoff rate
Zn	Old zinc panel, 40 years age	3.5
	General galvanized	2.7
	Zinc sheet (Cu, Ti)	2.3
	Pure zinc (99.97%)	2.1
Zn-Al	Spray-coated ZnAl 15	1.6
	Galfan	1.1
	Galvalume	0.8
Zn-P	Zn Quartz	1.3
	Zn Anthra	1.3
Zn-TC	Galvanized + chromate	1.4
	Galvanized + TOC	0.6
	Galfan + TOC	0.4
	Galvalume + TOC	0.3
Zn-top	Spray-coated Zn + topcoat	0.08
	Prepainted galvanized	0.07
Blank	Plexiglas surface	0.02

Calculations of the the total amount of zinc being released from unpainted galvanized steel on buildings and other constructions in Stockholm were made by Sörme et al. (2001b). They used figures published by Tolstoy et al. (1989) on the average surface of roofing and similar materials on buildings (1 m² / inhabitant) and on the average surface of other galvanized constructions, such as barriers, fences, poles etc. (2.33 m² / inhabitant) and based the calculation on the then actual figure: 711,000 inhabitants in Stockholm, as well as on the average zinc runoff rate of 3 g/m², y. This calculation gives a total annual zinc release from buildings and other constructions of 2.1 t + 5.0 t, i.e. a sum of 7.1 tonnes of zinc leaving buildings and infrastructure in Stockholm. The present estimate of the quantity of zinc being released from unpainted galvanized material surfaces in Greater Stockholm is well below earlier estimates. For example, Frise (1994) arrived at a range of 20-25 tonnes of zinc released per year in the early 1990s. A more detailed calculation, carried out by Landner and Lindeström (1998) came to a figure of 13.7 tonnes per year, a level which was about twice the now presented figure. A time-dependent downward trend in zinc corrosion and zinc runoff in Stockholm is plausible, since atmospheric SO_2 concentrations have decreased drastically from the mid-1980s, and SO_2 is a strong determinant of the corrosion/runoff rate.

In analogy with the investigations of the speciation and bioavailability of copper in the runoff water, the water-ligand computer model MINTEQA2 was applied to the zinc speciation question, as was the zinc-specific biosensor with bacterial strains of *Alcaligenes eutrophus*. The modelling approach predicted that 95-99.9% of all zinc in the runoff water at the edge of the roof would be present as free, hydrated Zn(II) ions. Depending on the pH and sulphate content, $Zn(OH)^+$ and $ZnSO_4$ (aq) could also be present. The zinc-specific biosensor likewise indicated that all zinc being released from the various zinc-based materials was bioavailable. In tests for growth inhibition with green algae, it was found that the ecotoxicity was predominantly goverened by zinc, i.e. the zinc in the runoff water was to a large extent bioavailable (Karlén et al., 2001).

The possible further fate of the runoff zinc was studied in a series of soil column experiments, in which artificial runoff water with 5 mg Zn/l added was allowed to pass through a well characterized soil from Belgium, and the soil retention capacity for zinc was followed for up to 3.5 years (Bertling et al., 2002c). The investigation was part of a large European industrial collaboration project aiming at increasing the knowledge on runoff rates, bioavailability and ecotoxicity of metals dispersed from zinc-based construction materials used in society.

When the artificial runoff water containing total zinc at a concentration of 5,000 µg/l had percolated through a soil core (55 g dry weight), only 60 µg Zn/l (1.2%) was left, and the zinc concentration of the percolate also included the naturally leached zinc from the soil. Thus, the zinc retention capacity of the soil, which is characterized as a low metal-retention soil, was about 99% and continued to keep this capacity even after three and a half years of constant percolation. The bioavailable fraction of the low amount of zinc remaining in the percolate was only about 34% of that in the fresh runoff water (Bertling et al., 2002c).

The potential of the zinc, primarily retained in the soil column, to become mobilised on treatment with artificial rain was also investigated. It was found that about 13% of the zinc in the top 1-cm layer was remobilised, but only 0.5% of the zinc residing in the middle and bottom layers of the core. The more immobile zinc apparently formed part of the zinc originally existing in the soil, since even after 3.5 years of continuous rainfall, the runoff zinc was not found in layers deeper than 3 cm from the surface. This finding led to the conclusion that a breakthrough of the zinc retention capacity of the investigated soil would not occur for at least 140 years per kg of soil (Bertling et al., 2002c).

Thus, if this soil is representative for urban and road-side soils, that are regularly exposed to runoff from zinc-containing material surfaces, it is suggested that the predominant part of the released zinc is retained in soils in the close surroundings of the buildings and other structures. In central urban areas with hard-made surfaces, where the runoff is almost immediately collected in stormwater sewers, the possibility of retaining the released metal is, of course, thwarted and dissolved zinc species may – to a larger extent – be transported to surface water recepients before adsorption and precipitation processes bring about a fixation of the mobile species.

4.1.5 Releases and flows of chromium and nickel

Although extensive investigations have been conducted on the dispersion rates of copper and zinc from material surfaces on buildings and other constructions containing these metals (see sections 4.1.1-4.1.4), relatively few studies have been devoted to the release of metals from outdoor building elements made of stainless steel. Recently, however, release rates of chromium and nickel from pickled and skin passed 304 and 316 stainless steel were first determined and reported after one-year field exposures in the urban atmosphere of Stockholm (Odnevall Wallinder et al., 2002b). Later on, the exposures continued for a period of 4 years, and the results were reported in 2003 (Odnevall Wallinder et al., 2003).

Traditionally, 304 stainless steels have been used for roofs and exterior façades on buildings in urban environments, and 316 stainless steels mainly for constructions in marine environments. However, the most common quality of stainless steel used today for roofing appications in both environments is 316 stainless steel. The average annual release rates of chromium and nickel from the two materials are as shown in Table 4.2.

Table 4.2. Annual release rates (mg/m²) of chromium and nickel from 304 and 316 stainless steels, exposed for four years to the urban atmosphere in Stockholm. After Odnevall Wallinder et al. (2003).

Type of stainless steel	Release of Cr	Release of Ni
304	0.2 – 0.6	0.1 – 0.5
316	0.2 – 0.7	0.3 – 0.8

The runoff rates of chromium and nickel from abraded metal surfaces increased significantly with decreasing pH of the rainwater, but were not directly influenced by rain intensity. It was also noted that surface conditions have a major influence on the release rate, which suggests a non-uniform corrosion attack (Odnevall Wallinder et al., 2002b). Immediately after release, chromium in the runoff is predominantly present as Cr(III) (>98.5%) and nickel as Ni(II) (>99%). The chemical forms change when runoff passes through different media, such as soil, where >94% of the two metals were found to be retained (Odnevall Wallinder et al., 2003).

The very minor releases of chromium and nickel from stainless steel used in the outdoor built-up environment in Stockholm for roofs and other building materials appears to be even smaller than was previously estimated, based on short-term atmospheric exposures of grade 304 stainless steel plates (Walterson, 1998). According to these earlier estimates, the runoff rate of chromium was 1.6 mg/m², y and the nickel runoff rate 1.4 mg/m², y. Based on these figures, Sörme et al. (2001b) calculated the release of chromium to be 0.6-0.7 kg/y and of nickel, 0.5-0.6 kg/y from a total surface of exposed stainless steel in Stockholm of 370,000-430,000 m². With the new, more accurate release data, chromium and nickel losses from roofs and constructions (stainless grade 316) would be 0.25 kg/y and about 0.3 kg/y, respectively. This would represent less than 0.03% of the total chromium emissions from goods in Stockholm and about 0.05% of the total nickel emissions from goods.

4.2 Case Study 2: Relative importance of the traffic sector for metal fluxes from the urban environment to aquatic ecosystems

The fate and effects in the aquatic environment of trace metals emitted primarily from road and street traffic in Stockholm were investigated by a team of researchers at the University of Stockholm. The team selected a number of water bodies in the vicinity of central Stockholm, where major outfalls of urban storm-water (mainly street runoff) are located, and sampled water, sediment and several species of invertebrates (in addition, transplanted zebra mussels were used). The rationale was to find out to what extent the emitted, traffic-related metals would be bioavailable to benthic animals and thereby constitute an ecological risk.

It was found that the levels of tungsten (W), lead (Pb), Zn, antimony (Sb) and Cu showed strong co-variation in the storm-water receiving areas; the relationship with W and Pb, metals dominating the pollution picture, was considered to support the existence of a significant influence of road/street traffic (however, other plausible sources of W emissions were not discussed).

The body-burdens of Cr, Cu, Ni and Zn in transplanted zebra mussels were determined at 9 different sites after in situ exposure for 6 weeks. No enrichment of Ni and a slight enrichment of Cu was noted in the mussels along a gradient from unpolluted areas to central Stockholm, while Cr and Zn were enriched 4.3 times and 2.3 times, respectively. A comparison of metal body-burdens in mussels exposed at a site downstream of Greater Stockholm with those of mussels from reference sites upstream of Stockholm, showed that almost no enhancement of Cr, Cu or Ni could be detected, while Zn was slightly increased. Thus, the method used to assess the contamination with bioavailable metal species in road/street-related emissions from the city of Stockholm could not demonstrate any impact of Cr, Cu or Ni, but a slight increase in the body-burden of Zn in the mussels. However, it is not clear whether this enhancement of Zn in the mussel tissues was caused by current emissions from Stockholm or by historical contamination that influenced the mussels through resuspension of older bottom sediments.

4.2.1 Difficulties to quantify the contribution of street traffic to trace metal fluxes

Road and street traffic is usually considered to be an important source of metal emissions to the environment. For example, in the series of monographs on metals published in the late 1990s, it was estimated (for the whole of Sweden) that the emission of copper from road and street traffic would be about 90 t/y, i.e. 44% of total copper emissions to the environment (L & L – Cu), the traffic emissions of zinc were about 500 t/y, i.e. 28% of the total (L & L – Zn), while the traffic emissions of chromium and nickel were 40 t/y (37% of the total) and 20 t/y (20% of the total), respectively (EW – Cr,Ni,Mo).

Corresponding estimates of traffic-related metal emissions for 1995 in the city of Stockholm (Bergbäck et al., 2001; Table 3.9) resulted in the following percent fractions of the total metal emissions from goods accumulated within the city: Cu – 38%; Zn – 62%; Cr – 99%; and Ni – 99%. It should be understood that a considerable part of the metal emissions from the road and street traffic in Stockholm originates from wear of road pavements.

Although the emissions from the various materials involved in street and road traffic may be rather accurately estimated, it is a considerably more difficult task to assess the fate of the metals emitted. This is due to the fact that the metal emissions primarily are in the form of dust particles, resulting from the wear of tyres, brake linings and road pavements and that a great (but variable) part of these particles may be transported away from the streets and roads as airborne particles and will settle either close to the roads or at longer distance depending on the actual weather conditions. Moreover, if the roads are surrounded by soft soil and various kinds of vegetation, the majority of the particles may be retained in this near-field zone. On the other hand, if street and road verges are sealed with concrete or asphalt pavements – and street sweeping is not carried out regularly – the metal-containing particles will, sooner or later, be washed into the storm-water sewer.

Depending on the type of sewer system, combined or separated storm-water and sewage system, the traffic-generated metal-bearing particles will be transported to the STP or with the separate storm-water sewer directly to the nearby lake, river or coastal water. Thus, of the initial estimate of traffic emissions of copper, 90 t/y for the whole of Sweden in the mid-90s, only some 10 t/y were supposed to end up in the aquatic environment, the rest being retained in soils along roads, in sewage sludge or in landfills (L & L – Cu). In the city of Stockholm, however, due to the large surface areas covered with hard, impermeable materials, it must be assumed that a relatively greater fraction of the metal emissions from traffic activities will end up in the sewers and eventually in either the sewage sludge or in the receiving surface waters around the city.

4.2.2 Bioavailability to aquatic organisms of traffic-emitted metals

In order to investigate the fate and effects in the aquatic environment of mainly the traffic-generated trace metal emissions in the city of Stockholm, Lithner et al. (2003) conducted a study during the fall of 2001 in some of the near-field storm-water-receiving water-bodies in the Stockholm area. The authors declare that in order to make "an extended ecological risk assessment, it is important to consider the *bioavailability* of toxicants, which are hitherto poorly known in the actual area. To improve the knowledge, biota were analysed for metals and POP in receiving waters, including Lake Mälaren, Saltsjön, and three small lakes."

The rationale behind the design of the study was that storm-waters in Stockholm often have high concentrations of nutrients, road-salts, metals and polyaromatic hydrocarbons (PAHs). In those parts of greater Stockholm, where the storm-water sewers are separated from the domestic waste-water sewers, the storm-water sewers usually empty in the closest surface water body, such as the eastern-most parts of Lake Mälaren as well as in some smaller lakes (Trekanten and Råcksta Träsk) which, consequently, are the water areas in Stockholm that are specifically influenced by storm-water, mainly transporting pollutants from road and street traffic. This is in contrast with the western-most parts of the Stockholm Baltic Sea archipelago (here called "Saltsjön"), where current outfalls from the STPs have a relatively strong impact. It should be noted that up till 1985-89, two STPs were discharging their effluents to Lake Mälaren, one (Eolshäll) had its outfall close to a site with the name of Klubbensborg and the other one (Bromma STP) discharged at Nockeby. It is highly probable that there are some remaining accumulations of pollutants in the sediments at these sites.

In order to investigate the impact of bioavailable metals and some organic pollutants (PAH, PCB and some chlorinated pesticides) possibly emitted with the storm-water to Lake Mälaren, zebra mussels (*Dreissena polymorpha*) of unit size were transplanted to 8 different sites in the lake, along an assumed pollution gradient from upstream reference sites to central Stockholm. In addition, a site in Saltsjön was also used. The mussels were exposed to the water at each of the 9 sites for 6 weeks, whereupon the animals were defecalised for 48 hours in natural (clean) water and then analysed for metals and some organic compounds, such as PAH. Analyses of metals also included pooled samples of the crustacean species *Asellus* *aquaticus* and midge larvae (*Chironomus plumosus*), which were caught in shallow water close to the sites where the mussels were exposed. Moreover, adjoining sediments were sampled and analysed as were surface water samples from all the sites.

The results of the analyses showed that the pollution was, in relative terms, dominated by tungsten, lead, zinc, antimony, copper, gold, silver and tin, in the order mentioned (Lithner et al., 2003). All these metals, except gold, silver and tin, exhibited a strong positive co-variation, which the authors concluded was an indication of a common source, most likely being related to road/street traffic. The main source of tungsten was supposed most likely to be car tyre studs made of tungsten carbide. That road traffic was the plausible source was confirmed by the strong co-variation between tungsten, lead and PAHs.

Some of the highlights among the results from the investigation can be summarized as follows:

- Total concentrations in water of the four metals of concern in the present report varied in the waters of Lake Mälaren, from the levels at the reference sites to the levels in central Stockholm as shown in Table 4.3. Concentrations in Saltsjön and in the smaller lakes exposed to storm-water from traffic-intense areas are also shown.
- There was no indication of any contamination of the water with chromium or nickel, neither in Lake Mälaren while approaching the centre of Stockholm nor in Saltsjön or in the smaller, storm-water-exposed lakes.
- Contamination of water with copper was relatively weak when moving from the reference areas in Lake Mälaren to the most storm-water-exposed part of the lake. Not even the input from STP effluents in Saltsjön did have a marked impact on the Cu concentrations in the water mass.
- Zinc concentrations were clearly enhanced in the most downstream parts of Lake Mälaren, compared with reference sites. This was also the case in Saltsjön (3-5 times higher levels) and in the smaller, storm-water-exposed lakes, where there seems to be about a ten-fold increase in total Zn concentrations in the water mass.
- A “Principal Component Analysis” (PCA) of all water data from Lake Mälaren clearly showed that the trace metals Cu, Pb, W and Zn were closely related, indicating that they have a strong co-variation, possibly depending on a common origin (probably traffic emissions), while Cr – and especially Ni – were negatively correlated with the other mentioned metals. The authors stress that post-glacial clays in the region are an important natural source for Ni (and some other metals).

Table 4.3. Total concentrations of metals (µg/l) in the surface layer of water in Lake Mälaren, in Saltsjön and in two smaller lakes in Stockholm during the fall of 2001. After Lithner et al., 2003.

Site	Cr	Cu	Ni	Zn
Ref. sites, Lake Mälaren (3 sites, 2 samples from each)	0.2-0.8	2.2-2.5	2.8-3.2	0.7-1.1
Downstream sites, L. Mälaren (5 sites, 2-3 samples from each)	0.1-0.4	2.6-3.9	2.8-3.0	1.5-5.2
Saltsjön (Baltic Sea) (1 site, 2 samples)	--	2.2-2.3	1.4-1.7	3.4-3.7
Smaller lakes, storm-water-exposed (2 sites, 1 sample from each)	0.4-0.6	2.7-4.1	1.3-1.5	8.4-14

- Concentration gradients for trace metals in surficial sediments are difficult to establish on the basis of reported data (few data points with very great variation).
- With regard to the main focus of the report, dealing with bioavailability and bioconcentration of trace metals by measuring their concentrations in invertebrates and in the surrounding water, the only results that can be deemed reliable, and therefore discussed in this context, are those derived from the systematic transplantation experiment with mussels (*Dreissena polymorpha)*. Metal concentrations in mussel soft tissues (after defecalisation) are given in Table 4.4.
- Metal concentration data on *Asellus* and *Chironomus* and on their close environment are very limited and were collected in a non-systematic and haphazard way. The reasons why this data is omitted from our discussion are specified in section 4.2.4.
- The average enrichment factor of the four metals in mussel tissue (downstream sites / reference sites) is low, about 1 (meaning no enrichment) for Cu and Ni, and about 2 for Cr and Zn. In fact, only metals such as W, Sn, Pb and Sb exhibited enrichment factors of 2.5 to 7, according to the evaluation made by Lithner et al. (2003).

Table 4.4. Concentrations of some metals (µg/g DM)in soft tissues of Dreissena polymorpha after exposure for 6 weeks on stones in baskets placed at 8 different sites in Lake Mälaren and one site in Saltsjön during the fall (August-October) of 2001. Figures in the table are ranges of mean values for different sites. After Lithner et al., 2003.

Site	Cr	Cu	Ni	Zn
Reference sites, L. Mälaren (3 sites, 1 sample from each)	0.8-1.8	9.9-13	29-41	140-180
Downstream sites, L. Mälaren (5 sites, 1-2 samples from each)	0.8-5.6	11-16	28-37	180-360
Saltsjön (Baltic Sea) (1 site, 1 sample)	1.0	10	47	240

- When comparing the lowest values in mussels from the downstream sites in Lake Mälaren with the mean value at the reference sites, no indication was found of any enhancement of metal concentrations in the mussels for any of the four metals. However, it is obvious that there are some pollution "hot spots" in eastern Lake Mälaren , where some enhancement of concentrations could be detected, especially with regard to Cr (4.3 times increase) and Zn (2.3 times increase).
- Comparing the body burden of metals in the mussels exposed in Saltsjön (brackish water) with the reference mussels, again, there was no real indication of increased contamination caused by the emissions from Greater Stockholm.
- A significant positive co-variation was established between total metal concentration in the water and metal concentration in the mussels (this was the case for Cu, Ni and Zn, but not for Cr). However, only a weak correlation remained, and only for Zn, when the calculation was based on dissolved metals in water (dialyseable metal), indicating that the relationship metal-in-water / metal-in-mussel primarily could be explained by the existence of metal-bearing food particles.
- A very strong positive co-variation was also demonstrated between mussel body burdens of lead and tungsten on one hand and several medium heavy PAHs on the other, which gives support to the hypothesis that the contaminants mainly originated from road traffic.
- The authors point out that the bioaccumulation factor (BAF), defined as the organism's concentration (of the substance of interest) divided by the

ambient level (here measured as total concentration in the water), is a complex function of the substance's bioavailability. They also stressed that it is necessary to distinguish between essential and non-essential metals when evaluating the toxicological significance of BAF. Many groups of aquatic organisms have the capacity of regulating the uptake of essential metals, although this regulation may increase the organism's demand for energy which has to be allocated from other life-supporting systems.

4.2.3 Level of contamination with bioavailable trace metals in the waterways of central Stockholm, compared to other waters

The investigation conducted by Lithner et al. (2003) provides a general idea about the degree of contamination with bioavailable trace metals in the inner waterways of the city of Stockholm, based on the analysis of zebra mussels (*Dreissena polymorpha*) exposed for 6 weeks to the local water quality in cages placed in the water column at a distance of 1 m above the sediment surface. It should be clearly recognized that the mussels were exposed to an unspecified mixture of water with suspended solids originating from current (autumn 2001) effluents, drainage water and natural runoff from the catchment, together with various contributions of "older material", first deposited in the sediments of each of the investigated sites and then – to a smaller or greater extent – resuspended into the water column during the period of exposure of the mussels. According to Broman et al. (2001), it is reasonable to assume, on the basis of earlier estimates, that roughly about 50% of the material settling to a sedimentation trap in the eastern part of Lake Mälaren during a given period is in reality resuspended bottom sediment. The sedimentation traps are usually placed at a greater distance from the sediment surface than the baskets containing mussels in Lithner's et al. (2003) investigation. As a consequence, the mussels in the baskets are exposed to suspended particles in the water originating to at least 50% (probably more) from previously settled material.

The resulting levels of contamination of the mussels with metals, representing a mixture of present-day and historical metal loading, has been compared with the metal loading of zebra mussels from some other water bodies in the world (Lithner et al., 2003), see Table 4.5.

Table 4.5. Concentrations of some metals (µg/g DM)in soft tissues of *Dreissena polymorpha* after exposure for 6 weeks in central Stockholm compared to metal concentration in mussels caught in some other lakes and rivers. Ref. data compiled by Lithner et al., 2003.

Water body	Pb	Cr	Cu	Ni	Zn
Central Stockholm	0.2-0.9	0.7-5.9	10-18	25-47	170-380
Great Lakes, N.America (15th and 85th percentiles)	0.8-4.6	4.2-10	14-34	18-33	98-160
Lake Ontario, S. part	1.8-6.5	3.3-5.3	5-26	5-13	160-310
Lake Geneva	0.5-1.2	0.8-3.2	16-42	20-45	120-190
Subalpine lakes, Italy	1.5-6.1	1.9-5.3	11-36	9-26	130-370
River Moselle	4-20	1-4	22-50	20-45	120-240
River Po estuary	2.3-3.9	3.5	18-21	14-16	130-160

As mentioned above, it should be remembered that the study of the level of contamination by road traffic-generated bioavailable trace metals in the waters of central Stockholm, carried out by Lithner et al. (2003), does not specifically describe the current situation, as it was in the autumn of 2001, but some mixed, partly historical situation, influenced to an unknown degree by older, most probably greater, discharges of metals and organic pollutants. These pollutants were incorporated in the bottom sediments over several years. Later on, the contaminated particles may have been resuspended in the water column and absorbed by the mussels transplanted to the site in question.

Taking this into consideration, it is interesting to note that – according to the data in Table 4.5 – the road traffic origin of the studied metals, as shown by the low concentration range of the traffic indicator lead, obviously is not confirmed. As a matter of fact, the lead concentrations found in the mussels, exposed to water and suspended solids in central Stockholm waterways, are the lowest in all of the identified water bodies where metal data on zebra mussels were given.

Chromium in mussels exposed in Stockholm showed a relatively broad concentration range, rather much corresponding to the levels found in lakes in southern Europe and in the southern part of Lake Ontario, but the Swedish data were lower than what was recorded in the Great Lakes over the period 1992-98 (O'Connor, 2002).

The levels of **nickel** in Stockholm tended to be on the higher side, compared to most other areas shown in Table 4.5 and, although they showed good correspondence with the data from Lake Geneva and River Moselle, they were clearly higher than in the Great Lakes in North America and in northern Italy. Lithner et al. (2003) explains the high nickel values in the mussels in Lake Mälaren by geological factors (Ni-rich glacial and post-glacial clays in the region).

Copper concentrations in the Stockholm material of mussels were low, compared to almost all the other water bodies, with the exception of some very low values in North American lakes. Even a comparison with the 15th percentile of the mussel data from the Great Lakes survey, 14 µg/g DM, supports the conclusion that copper concentrations were not enhanced in the mussels exposed in the central Stockholm area, in spite of the partial impacts of older emissions of copper from industrial facilities and STPs in central Stockholm. The relatively narrow concentration ranges for copper in mussels from most of the shown water bodies and, in particular, the rather uniform upper levels, is also an indication of the capacity of the mussels to regulate copper accumulation in their soft tissues.

On the other hand, zebra mussels may have a weaker capacity to regulate their body burdens of **zinc**, as shown by the relatively broad concentration range and by the strong positive relationship between total zinc in water and zinc in mussel tissue (Lithner et al., 2003). These authors assume that the high zinc concentrations in the mussels exposed in eastern Lake Mälaren is due to the high levels of zinc in sediments and suspended matter in this part of the Stockholm aquatic environment. This is most likely an effect of historical zinc emissions from industrial sources as well as zinc runoff from unpainted galvanized metal constructions.

4.2.4 Possible over-interpretation of bioaccumulation data – a critical review

If we go back to the stated objective of the investigations published by Lithner et al. (2003), as it was specified in chapter 2 of their report, we can recall that the authors emphasized the importance of knowing the degree of bioavailability of the pollutants in order to be able to make a correct analysis of the risk of biological effects. This is because "*bioavailability constitutes an important link between the environmental concentration and the risk of biological effects, being of central significance in the ecological risk analysis. In order to be able to assess the bioavailability it is often* *convenient to analyse organisms from the area with regard to the actual substances, and relate these to ambient concentrations, which preferably should be determined at the same time."*

Knowing that the authors are well aware of how to proceed in theory, and what strategy to choose when designing a field investigation, it is somewhat surprising to learn how it was done in reality:

- The only systematic and reliable part of all the bioavailability studies included was the experiment with transplanted zebra mussels (*Dreissena polymorpha*). The mussels baskets were placed at 9 different sites, all at a distance of 1 m above the sediment surface. One or two samples of six defecalised individuals with a composite weight of 20 mg DM from each site were subjected to chemical analysis. Thus, results from this part of the study are reliable and can be used in an evaluation of the presence of bioavailable metals (and organic substances) in the water. The major uncertainty in the experimental design used is that it was not possible to discriminate between contaminants currently released from the anthroposphere and historical contaminants residing in the top layer of the sediment, and periodically being resuspended back to the water column.
- The studies of additional animal species with the aim of broadening of the survey of bioavailable metals was, however, not fulfilling basic scientific requirements and their interpretability was therefore unacceptably poor. To get representatives of the littoral ecological niche, two different crustacean species were collected, *Asellus aquaticus* from two reference sites and one contaminated site in Lake Mälaren as well as from three smaller lakes, and *Gammarus pulex* from two sites in Lake Mälaren. Moreover, to reflect the soft bottom profundal habitat, midge larvae, *Chironomus plumosus,* were collected from three contaminated sites in Lake Mälaren and from two sites in small lakes. The sample sizes of *Chironomus* varied from 0.8 to 7.7 mg DM and the number of individuals from 6 to 55.
- The possibilities to make meaningful comparisons between sites, or between different media at the same site, were not facilitated by the fact that sampling of water for metal analysis or of particles in water were made on other occasions than the biological sampling.
- A great deal of the discussion in the report is devoted to the possible relationships between metals in the water mass and metals in crustaceans or midge larvae, in order to get a basis for calculating BAFs. However, it must unfortunately be concluded that most of this discussion has a very low scientific credibility, due to the fact that:

- the basic material is very limited, few sites and only one sample per site;
- samples of the same species from different sites are not comparable;
- there is no correspondence in time between samplings of water and biota;
- sampling of water and biota (if simultaneous) was made from different water masses, not necessarily having the same water quality.

In fact, even with respect to the transplanted zebra mussels, it must be questioned whether the calculated BAFs have any real meaning, because sampling of water was generally made twice at each site (at a distance in time of 1-2 months, and not necessarily at the same date as the mussel exposure ceased), sometimes showing that concentrations varied by a factor of 2 to 3, at least for Cr and Zn. Unfortunately, water was sampled from a water layer (0.1-0.2 m below the surface) quite separate from the water layer where the mussels were exposed (1 m above the bottom, i.e. at 4-5 m water depth).

4.2.5 Which conclusions from the study are justified ?

This quite large-scale study of metals and organic pollutants, conducted by Lithner et al. (2003) in Stockholm's inner waterways, has provided some results, which are scientifically reliable and represent new and important knowledge. Among these, it is pertinent to mention:

- A certain number of "new" metals (not previously studied as environmental metals) were confirmed as pollutants in Stockholm. The pollution was, in relative terms, dominated by tungsten, **lead**, **zinc**, antimony, **copper**, gold, silver and tin in the order mentioned.
- Strong positive co-variation was demonstrated to occur between lead, tungsten, zinc copper and antimony in water, which was interpreted as an indication of a common source, supposed to be related to road traffic. (However, the authors do not mention that there used to be an industry producing electric light bulbs, containing tungsten, close to the sampling and mussel exposure site called "Saltsjön".)
- Traffic pollution was furthermore assumed to be confirmed by the strong co-variation between tungsten, lead and certain PAHs in the transplanted zebra mussels. (However, the authors failed to explain how tungsten

from the very stable tungsten carbide used in the tyre studs can be released and taken up by the mussels.)

- Since the authors pointed out that pollutant levels in living organisms reflect exposure better than do ambient concentrations in water and sediment, and that the presence in biota clearly shows that a toxicant is biologically available, one of the main objectives of the study was to use data on metals and organic pollutants in biota as a basis for an environmental risk assessment.
- The only (possibly) reliable data on **Cr, Cu, Ni** and **Zn** in biota presented was the data series showing levels of metals in zebra mussels transplanted to 9 different sites in the waters around Stockholm, where the mussels had been exposed to a mixture of present and historical pollution for 6 weeks before analysis.
- No enrichment of Ni and a very slight enrichment of Cu was noted in the mussles when moving from reference sites in Lake Mälaren to the sites in central Stockholm, while the same comparison for Cr and Zn indicated a certain enrichment (4.3 times and 2.3 times increase, respectively).
- When comparing the body burden of metals in the mussels exposed in the brackish water downstream of Stockholm with the reference mussels exposed in Lake Mälaren water upstream of Stockholm, threre was no clear indication of increased Cr, Cu or Ni contamination, and a very slight increase in Zn concentration, possibly caused by the combined emissions from Greater Stockholm.
- A comparison of the levels of Cr, Cu, Ni and Zn in transplanted zebra mussels in Stockholm with the levels of the same metals in some other, comparable, lakes and rivers in the world, leads to the conclusion that the Cr and Cu levels in the Stockholm material did not differ (or was lower) compared to the ranges obtained for the reference areas. Ni levels in Stockholm tended to be higher than elsewhere, explained by the high natural background for Ni in the region of Lake Mälaren. Zn, finally, was slightly higher in the mussels exposed in Stockholm, compared to some – but not all – of the reference materials.
- The small enrichments of the here discussed metals in biota may not be directly related to any current emissions from road traffic – or any other current emissions from activities in Stockholm, because the mussels reflect the combined impact of current emissions and historical pollution, to which the mussels are exposed by means of sediment resuspension.
- Thus, although the study by Lithner et al. (2003) might be used to assess the environmental risk of W, Pb, Sb, Au, Ag and Sn, as well as of various PAHs and chlorinated organics, it is not scientifically justified to

164use the presented data for any risk assessment, nor any proposals on strategic measures, regarding Cr, Cu, Ni or Zn.

The authors have dedicated much effort to data presentation, discussion and conclusions based on the results of their attempts to calculate BAFs or on the whole section dealing with *Asellus aquaticus* and *Chironomus plumosus*. These parts of their report are not scientifically reliable and should be left without consideration.

4.3 Case Study 3: Metal fluxes from households to STPs, sludge and agricultural soils

One of the most important routes for trace metal transport from the anthroposphere to sensitive ecosystems and to human targets is via municipal sewers to sewage treatment plants and further on to sludge, which may be recycled to agricultural land and, thus, back to the crop/food production system. It is therefore important to identify the dominant sources of potentially harmful trace metals occurring in the sewage treatment process, to undertake a speciation of these metals and assess their availability for uptake into crops and their toxicity to soil micro-organisms. Several broad reviews of these problems have been published in the last few years, with a special focus on Cu.

The release of Cu from Cu pipes to the tap water in houses has attracted considerable attention in recent years, mainly due to the rising concern for Cu build-up in agricultural soils when Cu-containing sewage sludge is utilized as a soil conditioner. Based on both equilibrium calculations and chemical analyses, it was concluded that at the pH and alkalinity ranges that are common in Swedish drinking waters, $CuCO_3$ (aq) is the main dissolved inorganic copper compound, and CuO the main solid one. The concentration of dissolved cupric ions is, in general, much lower than the measured total dissolved Cu concentration. Other common Cu species in tap waters are various adsorbed forms on the surfaces of hydroxides and oxides and those occurring as organic complexes or bound to colloidal organic particles. In the drinking water of the city of Uppsala, with usually quite high total Cu concentrations, it was found that free cupric ions in solution amounted at 3-11% of the total Cu content. High Cu levels in this drinking water were usually related to high concentrations of *dissolved organic carbon (DOC), supporting the hypothesis on the formation of organic Cu complexes.*

Application of sewage sludge of "good quality" to arable lands, parks, forests or reclaimed areas may provide many agronomic benefits and contribute to solve the problem of nutrient and trace element deficiency. It is, however, important to stress that too high concentrations of trace metals and other elements or (organic) compounds may render the sludge less useful for application on arable land. Therefore, a set of limits for metals, including Cu, in sludges to be used for land application have been enacted both on the EU and the national level.

Long-term studies (up to 18 years) in several countries (e. g. in southern Sweden) were used to evaluate the impacts caused by yearly addition to arable land of Cu-containing sewage sludge (on Cu accumulation in the soil, on soil fauna and microbes, on crop yields and Cu-contents in crops). Based on such studies and on experiments with direct addition of dissolved Cu to agricultural soils, the conclusion was reached that the most sensitive sub-system is the microbial activity in the topsoil. As an overall conclusion, it was stated that an application rate of 1.4 kg Cu/ha, year with sludge would be a safe and acceptable load for Cu on most soils, where cereals are grown. A three times higher application rate, 4.2 kg Cu/ha, year, was not found to have any detrimental effects on crops or on the soil ecosystem, but a significant increase in soil Cu levels was demonstrated both in the topsoil and in the subsoil. Therefore, the latter loading rate cannot be considered as sustainable on the soil types investigated. A provisional level of acceptable Cu loading with sludge would, for most common types of agricultural soils, be somewhere in the range of 1.4 – 2.0 kg Cu/ha, year.

In the quality declaration of the sludge, with respect to the metal content, it is recommended to give it as milligram metal per kg of phosphorus (i.e. mg Cu/kg P), instead of mg Cu/kg sludge dry matter, because the main rational for using sludge in agriculture is to recycle phosphorus back to the soil (for more details on metal bioavailability in sludge see also section 5.5.7 below).

4.3.1 Origin of trace metals in STPs and in sewage sludge

The amounts of incoming trace metals to sewage treatment plants (STPs) in European cities vary a great deal depending on the age and technical standard of the infrastructure, but also on the general way society is organized. In the present context, we are going to focus on the metal levels in sewage sludge, and these are determined not only by the total amount of incoming metals with the raw sewage. In summary, the main factors influencing the levels of trace metals in sewage sludge are (Landner et al., 2000):

(1) the sources of incoming sewage and, in particular, the relative importance of industrial inputs, street run-off and domestic sewage;
(2) the physico-chemistry of the drinking water, influencing the corrosion rate of tap water pipes and, thus, the levels of copper and zinc in domestic sewage;
(3) the success of exisiting emission reduction strategies (e.g. reducing industrial inputs);
(4) the size of the treatment plant;
(5) the sludge treatment process itself.

In Stockholm , for instance, a range of emission management initiatives have achieved an important decrease in the metal levels entering the STPs over the last 25 years, especially with regard to copper. The decreasing tendency for copper is, however, not necessarily reflected in the copper concentrations measured in the sewage sludges because metal levels, expressed as sludge dry matter, depend to a large extent on sludge treatment practices. For example, the anaerobic digestion of a sludge will reduce its content of organic matter – in most cases - by 30 to 50%. This will automatically entail variability in sewage sludge data. For comparison purposes, it is therefore recommended to set sludge limits for metals in direct relation to the macronutrient phosphorous and thus express sludge limit values in mg metal/ kg P instead of mg metal/ kg sludge dry matter (Delbeke and Landner, 2000).

An approximate picture of the relative contribution of different sources to the metal loading of the STPs – and hence to the sewage sludges – in a city like Stockholm, with minor additions from industrial sources, can be obtained from Table 3.11. Especially in older parts of the city, there is a combined sewer system for stormwater and domestic sewage, which means that metals released e.g. in the street environment or from roofing and building materials will be transported to the STP together with the domestic sewage.

One of the important sources of metals in the incoming water to the STPs in Stockholm as well as in many other cities obviously is the tap water pipes and other parts of the plumbing system in houses, especially when these are made of copper. Therefore, many attempts have been made to quantify the rate of metal release and explain the exact mechanisms behind this release, in order to find appropriate counter measures. Recently, Linder and Taxén (2002) have made an in-depth examination of the problem by means of literature research and equilibrium calculations, based on the situation (water characteristics and service conditions, etc.) existing in Sweden, see below.

4.3.2 Assessment of the causes of copper release from tap water pipes

Corrosion products, released from materials used in manufacturing of pipes and other parts of plumbing systems (e.g. heat exchangers), will always be present to some extent in tap water, as long as metals are used for these purposes (Linder and Taxén, 2002). This applies to all metallic materials irrespective of alloy. However, the extent of metal release may differ widely between different materials and for different water qualities and service conditions.

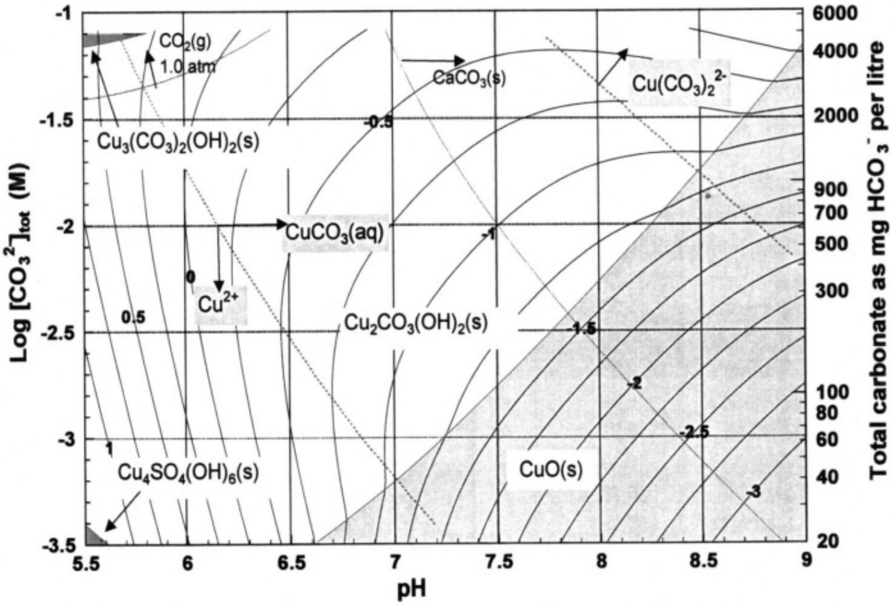
In particular, the release of copper from copper pipes to the tap water has attracted considerable attention in recent years, not only due to the possible impact on human health of ingested copper with the drinking-water, but even more so (at least in Sweden) because of the rising concern when copper-containing sewage sludge is to be utilized as a soil conditioner in agriculture. A large number of studies have been published, where the influence of the water quality (inorganic as well as organic water composition), the water flow regime, the age of the pipes, etc., on copper release to tap water has been investigated (reviewed by Linder and Taxén, 2002).

In older times, it was generally believed that elevated copper levels occurred in tap waters when the water was highly acidic, as was the case in many soft, untreated waters from private wells (Shull et al., 1960). However, in addition to this well-known influence of the pH-value, interest has later been turned also to the influence of inorganic carbon (carbonate) and natural organic matter (NOM) in the water. Obvious reasons for the interest in water alkalinity was, on one hand, the observation that higher copper contents were more often found in hard than in less hard waters and, on the other, that divalent copper may form soluble complexes with carbonate (see e.g. Mattsson, 1980).

Quite often, carbonate was discussed as a single factor influencing copper release from plumbing materials. However, high carbonate levels in the water often occur together with a high calcium content. In such waters, the pH had to be kept comparatively low to avoid undesirable calcium carbonate precipitation. Thus, a high carbonate content in the water can often coincide with comparatively low pH values and, therefore, the observation of elevated copper in hard waters may – to some extent – be a pH effect and not only a result of high carbonate content (Linder and Taxén, 2002). In fact, an examination of the relationship between pH and carbonate content in the waters of 310 Swedish municipal water works has revealed that the highest concentrations of HCO_3^- (>200 mg/l) clearly coincided with pH-values in the range 7.3-7.4, while HCO_3^- concentrations <100 mg/l were the rule at pH-values >7.6.

In the work by Linder and Taxén (2002), equilibrium calculations were carried out in order to examine the influence of various inorganic parameters on the copper solubility in a copper tube. These resulted in diagrams, which show the existing solids, the dominating dissolved cupric species and iso-concentration lines for dissolved divalent copper, including soluble complexes as functions of pH and content of inorganic carbon in the water. The diagram represented in Figure 4.1 shows the results when the thermodynamically stable solids copper(II)oxide, malachite, azurite and brochantite are present. Under these conditions, it was found that sulphate and chloride had no significant effect on the solubility of cupric copper. For the pH and alkalinity values usually encountered in Swedish drinking-waters, it is obvious that $CuCO_3$ (aq) must be the predominant dissolved inorganic copper compound and CuO the main solid compound. The concentration of dissolved cupric ions is, in general, much lower than the measured total dissolved copper concentration (Sundberg, 2002).

As will be further developed below, the deviation in concentrations of dissolved copper compounds from what can be predicted from the diagram in Figure 4.1, representing only inorganic copper species, is mainly attributed to NOM or colloidal effects.



Curved thin lines: **iso-concentration lines for maximum content of copper in solution as a function of pH and total carbonate content. The logarithm of the total concentration in mg /l of dissolved divalent copper is indicated with extra bold figures on every second line.**
Left-hand Y-axis: **logarithm of total carbonate content mol/l.**
Right-hand Y-axis: **total carbonate content as HCO_3^- mg/l.**
White field: **malachite limits the copper solubility.**
Left grey field in the lower corner: **basic copper sulphate (brochantite) limits the copper solubility.**
Left grey field close to the upper corner: **azurite limits the copper solubility**
Right grey field: **copper (II) oxide (tenorite) limits the copper solubility.**
Dotted lines (red): **enclose area where $CuCO_3$ (aq) is the dominating form of dissolved copper.**
Below left dotted line: **Cu^{2+} is the dominating form of dissolved copper.**
Above right dotted line: **Cu $(CO_3)_2^{2-}$ is the dominating form of dissolved copper.**
Solid line in upper left-hand corner: **saturation limit of CO_2 (g) at 1 bar.**
Solid diagonal line: **Saturation limit of $CaCO_3$ (s).**

N.B. Red dotted lines are those two lines in the figure running from pH 7.2 to the upper left corner, and from 400 mg HCO_3/l to the upper fifth quadrant, encompassing the solubility range where Cu^{2+} is the dominating form of dissolved Cu.

Figure 4.1. Results of equilibrium calculations for dissolved copper species, when all the thermodynamically stable solids are considered. Reprinted with permission from Linder and Taxén, 2002

Some of the additional main conclusions from the work of Linder and Taxén (2002) are summarized below:

- It has previously been assumed that $Cu(OH)_2$ (s) – a precursor of CuO (s) – is the only solid that controls the copper solubility at all the pH values and all the carbonate concentrations found in tap waters. However, the results of the actual equilibrium calculations revealed that, in fact, it is less likely that the $Cu(OH)_2$ model is generally valid for predicting the copper content in tap waters.
- The presence of NOM in the water can increase the equilibrium concentration of copper and also affect the kinetics of attaining equilibrium with solid corrosion products. Complex formation between copper ions and NOM in the water cannot be neglected, but it is not likely to be the only cause of very high copper concentrations.
- Copper may be adsorbed on substrates, both inorganic and organic, that have a colloidal nature. Tap waters contain various degrees of dispersed particles that may offer adsorption sites of both types. If copper is present in the water as dispersed small particles of corrosion products, this could explain some of the observed very high concentrations of copper.
- More copper can be kept in the water if instead of complex formation, the main role of the organic substance is to disperse small, colloidal, particles of cupric copper compounds.
- The copper release to drinking-water is not only a function of the water composition, but also of the flow in the pipes.
- In many cases, the most efficient way to decrease high copper concentrations in a drinking-water is to increase the pH. For hard waters with high carbonate content, the pH-increase must involve softening.
- It would be possible to find combinations of flow and stagnation periods, which are optimal for reaching an equilibrium condition with the lowest possible content of dissolved copper in a drinking-water. However, the optimal conditions may be somewhat different for waters with different composition.

With the above equilibrium calculations in mind, Sundberg (2002) examined the copper speciation in the drinking-water of the city of Uppsala, Sweden, where high to very high copper concentrations have repeatedly been recorded. The water composition after >12 h stagnation in a cold water copper pipe, more than 30 years old, was analysed once a year for 5 years. Some of the variables showed greater variation than the others; e.g. pH varied in the range 7.2-7.7, alkalinity between 225 and 326 mg/l and total copper in the range 1.5-2.4 mg/l (after flushing the pipe, the total copper was 0.5 mg/l). Free cupric ions in solution were measured by means of an ion specific electrode, and were found to vary between 0.05 and 0.25 mg/l (3-11% of the total copper content).

In an attempt to explain the great discrepancy between the concentrations of cupric ions and total copper in the drinking-water, Sundberg (2002) relied upon a few analyses of DOC in the water, which varied between 4.4 and 16 mg/l, and concluded that most of the copper released from the copper pipe was complex bound to the organic material. Based on the observation that the increase in total copper in the water after flushing was rapid; i.e. from 0.5 mg/l to about 2.5 mg/l in 5 h, Sundberg further speculates that the corrosivity of the water brings about a rapid copper release, but that the released copper is almost immediately bound to the DOC (since almost no carbonate scale is formed on the inner surface of the pipe) and that the corrosion process will go on until an equilibrium is reached between dissolved copper and DOC-complexed copper. When water is drawn and fresh water enters the tube, the whole process starts again.

The predominant role of organic ligands for keeping copper released from tap water pipes in solution is further corroborated by a German study, which showed that TOC-values of >2 mg/l in most cases would give total copper levels of 1-4 mg/l after 4 h stagnation in copper pipes (Priggemeyer et al., 2001). It was estimated that some 90% of the total copper in this water was bound to organic matter (Sundberg, 2002).

4.3.3 Use of sewage sludge as a fertiliser in agriculture

A sustainable agriculture must be based upon an ecologically sound management of organic and nutrient waste in society, including optimal recycling. This implies that the organic material and nutrients contained in the crops, after having passed through society, should as far as possible be recycled back to the agricultural system. Therefore, the management of sewage sludge in an economically and environmentally acceptable manner is one of the critical issues facing society today. The scope of this challenge is currently increasing within the European Union (EU), since the amount of sludge produced by STPs is rapidly increasing as a result of the obligations laid down in various EU Directives. On the one hand, priority is given to beneficial uses of sludge and to recycling phosphorus and other plant nutrients contained in the sludge back to the food chain, rather than disposal by landfilling. On the other hand, the presence of various contaminants in the sludge has initiated boycott actions by the farmers' associations in several countries. This refusal to accept agricultural land application of sludge is fuelled by growing apprehension in the food industry and by consumer organizations that contaminants in sludge may constitute a potential threat to human health and the environment (Landner et al., 2000).

In the beginning of the 1990s, the total amount of municipal sewage sludge disposed of in the 15 states being members of the EU was slightly more than 7 million tonnes (Mt), expressed as dry matter (DM). Today, ten years later, the total quantity of sewage sludge in the EU can be estimated at close to 10 Mt. In 1992, some 39% of the produced sewage sludges were used in agriculture, and it is estimated that, today, the fraction being disposed of in this way has not grown to more than 40-45%. The still relatively low share of sewage sludges produced that are used in agriculture is mainly due to the widespread reluctance of farmers to accept sludge as a safe fertiliser or soil conditioner. However, this trend is counteracted by ever stricter national regulations on sludge quality, which in fact have resulted in improved quality of sludges during the past few years. By means of a set of strict quality criteria for the sludge, combined with effective monitoring for compliance with the criteria, it has been possible to come to some agreements between the sludge producers (the sewage works associations) and the farmers' organizations regarding the use of specified qualities of sludge in agriculture.

4.3.4 Permitted trace metal loads to agricultural soils

The existing EU Directive regulating the use of sewage sludge in agriculture (86/278/EEC) stipulates the highest permitted loading rate for trace metals as follows: 12 kg copper, 3 kg nickel and 30 kg zinc per ha and year as mean value over a period of 10 years. However, due to increasing concerns, several EU member states have set national limits well below the EU Directive (see Table 4.6).

A recent EU initiative (Third Draft to the Working Document on the Revision to the Sewage Sludge Directive) intends to improve the present rate of recycling of nutrients and organic matter contained in sludge by broadening the scope of the existing legislation (EU Directive 86/278/EEC) and including the management of sludge in outlets such as silviculture, green areas and reclaimed lands. It is recognised that sludge should be used when there is an agronomic interest for the crops and/or soils. To ensure that the use of sludge is carried out in such a way as to minimise the risks of negative effects on humans and the environment, the EU is discussing concentration limits for metals and organic compounds. The Working Document proposes on one hand new limit values for the immediate future (short-term limits) and, on the other, some medium and long-term objectives for metals to be reached by 2015 (medium-term limits) and 2025 (long-term limits), respectively (Table 4.6). Although the proposals for medium- and long-term limits have later been removed from the EU Working Document on sludge (I. Schoeters, ECI, pers. info, 2004), these proposals are included in Table 4.6 for reference purposes.

Table 4.6. Permitted trace metal loads (kg/ha per year) in the EU – at present and proposed for the future – as well as in some EU Member States and in some other countries. After Landner et al., 2000; Delbeke and Landner, 2000.

Region/ country	Chromium	Copper	Nickel	Zinc
EU, Directive 86/278/EEC	-	12	3	30
EU, proposed short-term limits	3	3*	0.9	7.5*
(EU, proposed medium-term limits	2.4	2.4	0.6	6.0)
(EU, proposed long-term limits	1.8	1.8	0.3	4.5)
Sweden (from 2000)	-	0.3*	0.025	0.6*
Denmark	-	10	0.6	40
U.K.	-	7.5	3	15
Germany	-	1.3	0.3	2.5
France	-	3	0.6	9
Austria (Steiermark), arable land	-	1.25	0.25	5
Canada	-	370	4	36
USA	-	75	21	140

* The competent authority may decide to allow higher loading rate of Cu and/or Zn if shown that the soil is copper- and/or zinc-deficient and that there is a specific agronomic need for the crops.

In addition to regulating the trace metal loading rate for sludge on agricultural land, most countries, as well as the EU, have also set limits for concentrations of trace metals (and of some persistent organic compounds) in sludge for use on land. Examples of such maximum trace metal concentrations in sludges in the EU (today and proposals for the future), as well as in Member States, are given in Table 4.7. It should be noted that while most concentrations are given as mg/kg dry matter (DM), the proposed short-term limits in the EU are also given in terms of mg/kg P, because one of the major incentives for a farmer of using sewage sludge on his/her land is to make use of the phosphorus in the sludge as a fertiliser. A concentration expressed on the basis of the P content of the sludge is also a much more stable base for interpretation, since sludges produced according to various stabilisation techniques will exhibit greatly varying dry contents. This means that relating the metal content to the DM content of the sludge will make it almost impossible to standardize the sludge quality.

Table 4.7. Limit values for some trace metals in sewage sludge (mg/kg DM, or when indicated, expressed as g/kg P) for use in agriculture. Values are from the EU – at present and proposed for the future – as well as from some EU Member States and from some other countries. After Landner et al., 2000; Delbeke and Landner, 2000.

Region/ country	Chromium	Copper	Nickel	Zinc
EU, Directive 86/278/EEC	-	1,000-1,750	300-400	2,500-4,000
EU, proposed short-term limits	1,000	1,000	300	2,500
d:o, expressed as g/kg P	25	25	7.5	62.5
(EU, proposed medium-term limits*	800	800	200	2,000)
(EU, proposed long-term limits*	600	600	100	1,500)
Sweden** (from 2000)	100	600	50	800
Denmark	-	1,000	30	4,000
Netherlands	-	450	70	1,400
Germany, soil pH 5-6.5	-	800	200	2,000
Switzerland	-	600	50	2,000
Austria (Steiermark)	-	500	100	2,000
Canada	-	-	180	1,850
USA, high quality	-	1,500	420	2,800

* Medium- and long-term limits removed from latest Working Document
** Mean value over a 7-year period

In order to get the proposed limit values into perspective, it would be interesting to compare these values with the weighted average metal concentrations in sewage sludge as reported by the EU Member States for 1999 (all expressed in mg/kg DM) : Cr – 73 ; Cu – 330 ; Ni – 36 ; Zn – 811 . As a matter of fact, it can be noted that the average metal levels in authentic sludge in 1999 were all well below the earlier proposed long-term limits for these four metals.

4.3.5 Sustainable trace metal loadings to agricultural soils

All the here discussed trace metals are known to be essential trace elements or micronutrients, needed for optimal growth and development of microorganisms, plants, animals and humans. (in the case of nickel, it is considered to be an essential element at least for some living organisms). In spite of the fact that chromium, copper, nickel and zinc – to various degrees – are essential nutrients, they may all become toxic at elevated levels. Therefore, limits for the exposure of man and the environment to these elements need to be based on a scientific evaluation of potential short and long term risks, both with regard to deficiencies and toxic effects. Such an evaluation needs to recognise the potential benefits and potential harm caused by application of trace metal-containing sewage sludge to agricultural land and indirectly, the benefits and risks for crops, animals, and human beings.

In order to recommend a sustainable trace metal loading rate with sewage sludge, regulations need also to ensure the long term quality of the soil. The accumulation of metals in agricultural soils, to levels that may pose future threats to crop cultivation or ecosystem functioning, therefore need to be avoided.

Natural trace metal levels in soils depend on the natural geological and physico-chemical characteristics of the soils. Total concentrations of trace metals in top-soils of agricultural land in European countries obviously show a considerable variation from region to region and even from place to place or field to field. Therefore, all organisms have developed mechanisms that allow them to regulate their internal levels of essential metals, within certain limits: at low trace metal exposure levels, intake rates may be increased while at high metal levels elimination rates may be enhanced. These so-called *homeostasis mechanisms* are however only functional within a specific optimal concentration range. Outside this range, deficiency symptoms or toxic effects may be observed. Considering the wide range of naturally occuring trace metal levels, different living organisms have developed different specific metal requirements as well as different optimal concentration ranges.

4.3.5.1 Trace metal deficiency symptoms and how to correct them

In agricultural practices, the natural environment is modified. Large biomasses of crops are cultivated and elevated concentrations of bioavailable trace metals in the soil are needed to sustain agricultural yields. In particular, copper deficiency has indeed been noted in a wide variety of agricultural soils world-wide (IPCS 1998), but also zinc deficiency may occur in specific soils, preferentially where antagonistic nutrient interactions exist. In Europe, at least 30 million hectares of agricultural land are cultivated with crops, whose growth and yields are known to be highly susceptible to copper deficiency. Eighteen million hectares of cultivated soils in Western Europe (equivalent to 19% of arable land) are believed to be deficient in bioavailable copper (Shorrocks and Alloway, 1985).

To compensate for decreased crop yields due to copper deficiencies, it is a common and necessary practice in agriculture to replenish the copper content of soils with copper-enriched fertilisers or copper sulphate amendments. In Sweden, for example, 34 tonnes/year of copper are added to agricultural soils via copper-enriched fertiliser to correct copper deficiencies. Additionally, 28 tonnes of copper are added through sewage sludge applications and 46 tonnes through manure application (L & L – Cu, 1999).

4.3.5.2 Application of sewage sludge to soils – risk of metal toxicity to soil ecosystems

The use of sludge in agricultural practices should be carried out responsibly to minimise any potential risks to the health of humans, animals, plants and soil microorganisms. A critical factor in assessing the risks of trace metal deficiency and/or toxicity is the bioavailability of the metals. Trace metals in the soil are subjected to a variety of processes limiting their mobility, such as sorption, complex binding and precipitation/flocculation. These processes, which are described in further detail in section 5.5 of this report, leave only a limited part of the total trace metal content, present in sewage sludge and soils, available for uptake by the organisms (e.g. Sauvė et al., 1998; Sauvė, 1999; Allen, 1999). Only this fraction of the metal in question has the potential to elicit effects (beneficial or adverse) on the organisms. As a consequence, in order to adequately evaluate the risks for soil organisms, it is extremely important to consider the bioavailability of the actual metals in sewage sludge (compared to the soluble metal salts, for instance) and soils (compared to hydrocultures for instance).

4.3.5.3 Long-term field studies – effects of sewage sludge application to soils

In various studies where trace metal-containing sewage sludge has been applied to agricultural land, no reduction in crop yields nor any adverse phytotoxic effects have been demonstrated, even after long-term applications of high quantities of sludge. However, long-term, realistic field studies of the consequences of sewage sludge application, where different aspects, such as crop yield, nutrient status of the soil, metal accumulation in the soil and in the crops, have been thoroughly studied over periods of up to 20 years, are still rare in the literature. For this reason, it was considered pertinent to give a relatively detailed summary of such a study, carried out in Sweden during the years 1981 – 1999. The study was initiated by a group of regional organizations in Scania, southern Sweden, representing nine municipalities, the farmers, the sewage sludge producers and research institutions at the Technical University of Lund. Financial support was given by the municipalities, the waste development company and VA-forsk (Water and Sewage Research foundation) and cooperation has also been established with the University of Agriculture, Uppsala. A series of reports from these studies have been published over the years, unfortunately, all in Swedish language, e.g. Andersson and Nilsson (1992; 1993, 1996 and 1999); Andersson (2000).

The general experimental scheme included a complete factorial of three rates of sludge application: A – no sludge; B – 4 t DM per ha every 4th year; and C – 12 t DM per ha every 4th year, and three rates of mineral fertilisation: 0 – no mineral fertiliser; 1 – half recommended addition of NPK (mineral fertiliser containing nitrogen, phosphorus and potassium); and 2 – full recommended addition of NPK (related to the actual crop). The timing and the crop rotations used during the experiment are shown in Table 4.8. The study was set up at two different farms in Scania, one close to the city of Lund (Igelösa) and the other close to the provincial capital, Malmö (Petersborg):

Table 4.8. Experimental scheme for studies of the long-term consequences of sewage sludge applications on agricultural land in southern Sweden. After Andersson, 2000.

Year	Application	Crops, Igelösa	Crops, Petersborg
1981	Sludge: 0, 4 or 12 t/ha	winter wheat, sugar beets	winter wheat, sugar beets
1982-85		spring wheat, oats	barley, winter rape
1985	Sludge: 0, 4 or 12 t/ha		
1986-89		winter wheat, sugar beets spring wheat, barley	winter wheat, sugar beets barley, winter rape
1989	Sludge: 0, 4 or 12 t/ha		
1990-93		winter rape and wheat sugar beets, spring wheat	same as above
1993	Sludge: 0, 4 or 12 t/ha		
1994-97		peas, winter rape, winter wheat, sugar beet	winter wheat, sugar beets spring wheat, winter wheat
1997	Sludge: 0, 4 or 12 t/ha		
1998-99		spring wheat, barley	sugar beets, spring barley

Readily dissolved (in ammonium lactate solution) plant nutrient levels in the two soils were:

- Igelösa: P – 90 ; K – 114 ; Ca – 4150 ; Mg – 100 (all expressed as mg/kg). pH was 7.0
- Petersborg: P – 110 ; K – 89 ; Ca – 1950 ; Mg – 70 (in mg/kg). pH was 6.8.

It should be noted that the recommended sludge application rate to agricultural soils (by Swedish EPA) is 1 t of DM per year, i.e. 4 t every 4th year. Application rate C, thus is a 3-fold excess.

The sewage sludge was taken from two different STPs, in Lund and Malmö, respectively, and the metal contents on the five occasions of application are shown in Table 4.9.

Table 4.9. Ranges of metal concentrations (mg/kg DM) in the sewage sludges used in the field experiments at Igelösa and Petersborg. After Andersson, 2000.

Origin	Cd	Cu	Cr	Ni	Zn
Lund (1981-97)	1.3-3.0	650-1700	28-210	13-110	600-1100
Malmö (1981-97)	1.7-3.5	1030-2000	29-410	25-30	660-1000
Guidelines, before 1995	4	600	150	100	1500

As can be seen from Table 4.9, the concentrations of at least copper and chromium in the sludge were clearly higher than the then existing guideline values. Considering that the amount of sludge applied to the soils were up to 3 times greater than the normal application rate, it is obvious that at least the influx of copper to the soils was abnormally high.

During a great number of years, the metal concentrations in the soil were measured (as total metal) and, thus, the impact of the sludge application could be followed by comparing the series C0 (12 t of DM sludge per ha every 4th year) with A0 (no sludge addition). The results of these analyses in the topsoil are shown in Table 4.10.

Table 4.10. Trace metal total concentrations (mg/kg DM) in the topsoil at Igelösa (IG) and Pertersborg(PE) in 1981 and at later stages just before a new sludge application. After Andersson and Nilsson, 1999; Andersson, 2000.

		Copper		Chromium		Nickel		Zinc	
Year	Ser.	IG	PE	IG	PE	IG	PE	IG	PE
1981	All	12	12	--	--	14	8.2	70	49
1986	A0	11	9.3	40	21	8.5	7.1	49	46
	C0	19	9.7	46	21	9.5	7.4	65	47
1989	A0	11	10	19	12	11	8.5	48	45
	C0	18	14	20	12	13	8.3	58	46
1993	A0	14	14	24	14	13	8.4	54	43
	C0	24	20	26	13	14	8.1	72	41
1997	A0	10	9.6	22	14	13	8.6	56	45
	C0	23	15	23	13	13	8.3	66	44
1999	A0	12	11	28	16	14	9.4	55	46
	C0	36	22	30	14	15	9.1	68	48

A = no sludge; C = 12 t DM of sludge per ha every 4th year; 0 = no mineral fertiliser

As will be seen from Table 4.10, the copper concentration in the topsoil at Igelösa has increased two to three times (from 12 to 23-36 mg/kg DM) as a result of the total addition of about 75 kg Cu/ha with sewage sludge over the period of 18 years. In spite of the fact that the copper addition to the soil at Petersborg was somewhat greater (about 84 kg/ha), the increase in total copper in the topsoil at this site was about two-fold or less (from 10-12 to 20-22 mg/kg DM).

Also the zinc concentration in the topsoil at Igelösa, but not at Petersborg, appeared to rise a little as a result of the sludge application. However, also the Igelösa series indicated that only small changes occurred. As far as chromium and nickel are concerned, no changes in their total concentrations were recorded in the sludge-amended soils.

Among the collected data from the year 1999, there is also a series of metal concentrations in the sub-soil (30-60 cm depth), allowing a similar comparison as the one made for the topsoil, see Table 4.11.

Table 4.11. Trace metal total concentrations (mg/kg DM) in the sub-soil (30-60 cm) at Igelösa (IG) and Pertersborg(PE) in 1999. After Andersson, 2000.

		Copper		Chromium		Nickel		Zinc	
Year	Ser.	IG	PE	IG	PE	IG	PE	IG	PE
1999	A0	9.1	8.9	20	17	14	13	49	42
	C0	13	10	19	18	13	14	51	43
1999	A2	8.8	8.4	19	16	14	11	47	43
	C2	17	9.2	22	13	13	9.7	59	39

For explanation of A, C and 0, see Table 4.10; 2 = full recommended addition of NPK

It appears that after 18 years of sludge application to the soil, there was a slight rise in the copper concentration in the sub-soil at Igelösa, both in the series without (0) and with (2) addition of mineral fertilisers. An interesting finding is that a similar rise in copper content was almost inexistent in the sub-soil at Petersborg, although the rise in copper in the topsoil was smaller at Petersborg than at Igelösa. The question therefore is: where did the 84 kg of copper per ha that were spread over the soil at Petersborg end up, since it was not reflected in a clear rise in copper content in the sub-soil at this farm ? A possible conclusion might be that local variations (size and treatment of plots, slope, water infiltration, etc.) and difficulties to get quite representative samples make it almost impossible to obtain a consistent picture from the relatively limited sampling programmes used. Another pertinent question is, of course: to what extent do the metal concentrations in the crops change as a result of sludge application to the soils at an application rate three times greater than normal ?

Metal analyses of the crops were conducted almost every year during the experiment and these enabled conclusions to be drawn on whether or not there were any changes in the metal uptake in the crops as a result of the sludge application. The conclusions presented by the authors (Andersson and Nilsson, 1993) after the first decade of the experiment were the following:

- *On average, there was no difference between sludge treated and non treated units in the metal content of crops.*
- *The copper concentrations drop at increased sludge application, if there is no simultaneous addition of mineral fertilisers.*
- *Also the levels of chromium drop at higher sludge additions, if no mineral fertiliser is added. However, the combination half of the*

recommended mineral fertiliser addition combined with a triple dose of sludge causes an increased chromium level in the wheat grain.

- *There was no effect on the nickel content of the grain when only sludge was added, but together with half the recommended mineral fertiliser addition, the nickel content of the grain increased considerably.*
- *No tendency to change whatsoever was found for zinc in the crops.*

In the (so far) last report (Andersson, 2000), additional data on metals in the crops was published. This data, regarding metals in spring wheat grains and sugar beets from 1998 and in spring barley grains from 1999, is reproduced in Table 4.12.

The results shown in Table 4.12 indicate that the metal contents in crops change relatively little on addition of sewage sludge to the soil, which occurred last time in 1997. However, there was a tendency to enhanced concentrations of copper in sugar beets and in barley grains at Petersborg, especially when sludge was applied in combination with mineral fertilisers. A similar increasing tendency was found for nickel and zinc in sugar beets at Petersborg, both with and without mineral fertilisers added.

The effects of sludge application on the crop yields was followed during all years of the field experiment. If was found that the sludge had a considerable beneficial effect on the yield of all crops, irrespective of the amount of mineral fertiliser added. When the sludge application rate was 3 times the normal (series 2) the percent increase in yield was in the range 17-29% , depending on whether it was winter wheat or rape, spring barley or wheat or sugar beets (Andersson and Nilsson, 1993). In the years 1998 and 1999, the harvest in series C2 (three times the normal sludge amount and normal dose of mineral fertilisers) was compared with that in the series A2 (considered as the most normal cultivation scheme), which was set at 100%. It was found that the harvest of spring wheat in 1998 in the series C2 was 108%, and that of sugar beets 105%. A similar comparison with the yields in 1999 gave, for spring barley at Igelösa, 141%, and for the same crop at Petersborg, 103% (Andersson, 2000). If the beneficial effect of the sludge addition on the tonnage of harvest is expressed in monetary terms, it can be concluded that every tonne of sludge applied to the soil had an average yield enhancing effect corresponding to 400-850 SEK (43-92 €) per ha and year.

Table 4.12. Metal contents (mg/kg DM) in the crops harvested in 1998 and 1999 at the two farms Igelösa and Petersborg, after several years of sewage sludge application. After Andersson, 2000.

Year	Crop	Farm	Series	Cu	Cr	Ni	Zn
1998	Spring wheat	IG	A0	4.6	0.35	0.22	31
			C0	4.4	0.13	0.22	33
			A2	4.3	0.32	0.16	25
			C2	4.1	0.20	0.18	30
1998	Sugar beets	PE	A0	3.6	0.33	**0.20**	**12**
			C0	3.6	0.34	**0.39**	**18**
			A2	**3.5**	0.36	**0.22**	**12**
			C2	**4.1**	0.41	**0.37**	**19**
1999	Spring barley	IG	A0	5.9	0.28	0.15	39
			C0	5.5	0.37	0.15	36
			A2	4.9	0.24	0.12	26
			C2	4.7	0.26	0.13	31
1999	Spring barley	PE	A0	**5.0**	0.48	0.18	18
			C0	**5.4**	0.42	0.20	20
			A2	**4.3**	0.46	0.15	17
			C2	**4.9**	0.35	0.16	20

In 1990/91, a special study was conducted at the two farm sites Igelösa and Petersborg to examine the impact of sludge addition (cumulative Cu input: 13 - 40 kg/ha) on reproduction and growth of earthworms (*Allolobophora chlorotica*). The overall conclusion was that sludge application, at the rates used in the experiment, had no negative effects on the worms (Andersson and Nilsson, 1993). The changes observed in the earthworm population were mainly positive. For instance, the sludge application appeared to be beneficial on the average production of cocoons per individual during 12 weeks, a measure of reproduction success in this species. No toxic or beneficial effects were found on the growth of juvenile individuals.

Samples were also taken for microbiological tests in the years 1990 and 1994 in order to evaluate the impact of the sludge addition on the soil microbiology. No direct negative influence on the microorganisms caused by the sludge addition was detected. However, the sludge addition may have produced some indirect effects on soil microbial activity, due to the changes in soil pH and content of organic matter. The only type of microbial activity that was affected at the highest sludge application rate was the heterotrophic nitrogen fixation, especially at the Petersborg farm (Andersson and Nilsson, 1993). It may be pointed out in this context that the ecological role of free living nitrogen fixing bacteria in the soil is rather small, since the quantities of nitrogen fixed are limited to a few kg of nitrogen per ha and year (Smolders, pers. comm.).

Finally, the positive impact on the nutrient status of the soil was also considerable: the phosphorus content of the soil was markedly enhanced at both farm sites as a result of the sludge addition and the content of organic matter in the soil was clearly improved (Andersson, 2000).

Thus, many of the desirable, beneficial effects of sludge recycling to productive soils were obviously obtained in this field experiment. It also appears that the potentially harmful effects of using sewage sludge as a soil amendment were limited. At least in this 18-year field study, it could be noted that using the recommended sludge application rate (1 t of DM/ha and year) and with most metal contents (notably except copper) below the official limits, the observed negative effects were marginal; maybe less important than the benefits of using the sludge.

4.3.5.4 Conclusions regarding sustainable trace metal loadings to agricultural soils

In many cases, man-made, persistent organic contaminants or metals such as cadmium, lead and mercury are considered to be the critical constituents in sewage sludge, determining if the sludge can be used at all as a soil amendment, or setting the limits for how much sludge can be applied to agricultural land, where human food is produced. However, when focusing on the four metals being treated in this report, chromium, copper, nickel and zinc, it is usually the copper content of the sludge that is the question of concern. If it is scientifically correct to assign a particularly high environmental and health risk specifically to copper in the sludge can certainly be debated.

For the moment it is quite clear that politicians, environmental authorities and NGOs in several countries tend to focus specifically on the copper content of sludges used in agriculture. Therefore, we shall briefly examine the issue of sustainable use of sewage sludge in agriculture in the perspective of the sludge as a vehicle for copper to soils.

A great number of studies and tests of adverse or toxic effects of copper in soils have come to the conclusion that the most sensitive subsystem is the microbial activity in topsoils (cf. chapter 7). Therefore, in the examination of the impact of copper on soils, we are going to focus on soil microbiologal function.

Thus, it has been deemed useful to elucidate the toxic effects of soluble copper added to soils on some important microbial processes, which may reflect a "worst case" situation. To this end, Torstensson and Johansson (1997) treated three soils in the region of Uppsala, Sweden, with TOC contents of 2.1, 2.5 and 4.5%, respectively, with cupric sulphate, applied on a carrier consisting of pure quartz sand, at the rates 3, 6, 8, 10, 12, 1,200 and 12,000 kg Cu/ha. The microbial functions measured were basal and substrate-induced respiration, nitrogen mineralization capacity, as well as potential nitrification and denitrification activity. The functions most sensitive to the copper application were – as expected – those related to nitrogen metabolism, and in all three soil types, denitrification activity turned out to be particularly sensitive.

The results, in summary, showed that a copper loading of 12 kg/ha, applied as copper sulphate, had a clear adverse effect on the microbial denitrification activity. At lower copper loadings (between 3 and 10 kg Cu/ha) no such effects were observed. Other microbial activities were unaffected even at much higher copper loading rates (Torstensson and Johansson, 1997).

Thus, copper sulphate amendments up to 10 kg Cu/ha did not have any effect on one of the most sensitive ecological endpoints. Considering the fact that copper added as sewage sludge is bioavailable to a lower extent than copper sulphate, one can assume that an addition of 12 kg Cu/ha applied as sewage sludge is not expected to have any adverse effects on the ecological function of agricultural soils. This appears to be a safe conclusion, since it was recently shown that effects of metals on microbial denitrification, although pronounced at 1 day after spiking, disappear almost completely if the soil is incubated for 2 months after spiking and prior to testing (Smolders, pers. comm.).

In order to recommend a sustainable copper loading rate, one also needs to ensure the long term quality of the soil. In this context, it is pertinent to recall that in various EU Working Documents dealing with the use of biowaste or sewage sludge on agricultural land, it is stressed, e.g., that "soil protection is deemed to imply a steady state condition for heavy metal inputs to soils that would guarantee that total background concentrations are not dramatically increased in the long term". The accumulation of copper in soils, to levels that may pose future threats to crop cultivation or ecosystem functioning, therefore needs to be avoided. Several investigations have indeed reported important accumulations of copper in soils at elevated copper loading rates (e.g. Ohlsson et.al, 1997).

The above referred long-term field studies in southern Sweden (Andersson, 2000) came to the conclusion that after 18 years, there were no significant increases in copper levels in cereal crops (but a slight increase in sugar beets after sludge application together with mineral fertilisation) and that there were no detrimental effects on crop yields. Copper accumulation in these soils (pH 6.8 – 7.0) was quite noticeable at an application rate of sludge-bound copper of 4.2 kg Cu/ha and year, but was only marginal at application of 1.4 kg Cu/ha, year. After 12-16 years of application at this lower rate, the final copper levels in the topsoil were 13 to 20 mg Cu/kg soil DM, and thus well below the 50 – 140 mg/kg DM soil used in the current EU Directive (86/278/EEC), or the 50 mg/kg DM, at soil pH of 6-7, proposed in the third draft Working Document (or the 40 mg/kg DM stipulated as limit value in Sweden). This relatively modest level of copper accumulation, in addition to the absence of any detrimental effects over the short-term or long-term, allows us to conclude that sludge application at 1.4 kg Cu/ha, year appears to be sustainable for agricultural soils of the type used in the referred experiments, and is not likely to have any adverse effects on crops, animals, humans, or ecosystem functions.

The higher copper application rate used in the long-term field experiment in southern Sweden, 4.2 kg Cu/ha, year, did not cause any obvious detrimental effects on crops or on the soil ecosystem, but the above mentioned significant copper accumulation that was demonstrated to occur both in the topsoil and in the subsoil of the investigated soil types calls for a special consideration. Since it has not been shown that steady-state conditions have been reached for copper in soil on the higher loading rate (4.2 kg/ha, year), it is not possible to characterize this as a sustainable loading rate. On the other hand, it seems reasonable to conclude that the lower rate, 1.4 kg Cu/ha, year, really represents a sustainable level of copper loading to the actual types of agricultural soils, if the loading takes place with copper incorporated into sewage sludge. The real limit value for sustainable copper loading to these types of agricultural soils obviously is somewhere in between the two levels, 1.4 and 4.2 kg Cu/ha, year, although it must be kept in mind that different soil types may have different maximum levels of sustainable loading with copper as well as with other trace metals.

Sewage sludge contains valuable nutrients and essential minerals, including copper and zinc. The sludge can be used beneficially in a wide range of applications, such as on forage crops, pasture lands, arable lands, parks, forests, silviculture (pine forests), and land reclamation projects. The recycling of sewage sludge of “good quality” may provide an agronomic benefit to crops and be a sustainable solution to the problem of nutrient deficiency in arable soils (phosphorus, nitrogen, copper, zinc). It is, however, important to mention that other elements or compounds (such as persistent and toxic organic compounds) than copper may, in some cases, render the sludge less useful for application on arable land.

For copper, as a constituent of sewage sludge, an application rate of 1.4 kg Cu/ha, year up to perhaps 2.0 kg Cu/ha, year appears to be a safe and, thus, acceptable load limit on most common types of agricultural soils. Further reductions in loading rates are not scientifically founded when considering the latest scientific information. Too low target values will misinform the public and are neither compatible with the scientific evidence available, nor with the current use of copper as a necessary nutrient in agricultural practices.

When specifying and evaluating the levels of copper – and other metals – in sewage sludge it is recommended to express the metal concentrations in direct relation to the macronutrient phosphorous. Thus metal concentrations should rather be given as mg metal/ kg P, instead of mg metal/ kg sludge DM as has been common practice earlier.

4.4 Case Study 4: Metal fluxes from mine waste to rivers – Falun Copper Mine

Mixed sulphidic ores (mainly containing Fe, Cu, Pb and Zn) were mined for more than a millennium at the Falun Copper Mine, central Sweden. The exploitation was conducted in industrial scale for 450 years, until the operations ceased in 1992. The great importance of the Falun Mine for the technical, economic, social and political history of Sweden and Europe was recognized by the designation of the Mine and parts of Falun city as a World Heritage Site by UNESCO. But the long history of heavy environmental pollution from the mining and metallurgical activities is also unique and deserves recognition and assessment. As an element of the large-scale "Falun Project" aiming at a general clean-up of the Falun region and near-by River Dalälven, a book entitled "The Environmental History of the Falun Mine" was prepared by L. Lindeström and published in 2003.

According to current estimates, a total of 6 Mt of SO_2 *and 15 kt of Cu was emitted to the atmosphere, while 0.5-1.0 Mt of Cu, Pb, Zn and Cd were discharged to the surrounding forest soils and watercourses during the operations in Falun. In the small stream flowing through the city of Falun, the total concentration of Cu was enhanced about 140 times (reaching 70* *μg/l) and that of Zn about 1,000 times (reaching 2,000 μg/l) relative to reference levels upstream of Falun, even after purification of the mine water had been initiated.*

The assessment of the Falun environment showed that the soils and the terrestrial ecosystems surrounding Falun had recovered to a remarkable degree during the 20th century. The aquatic environment had much less time to respond to pollution abatement measures, since the treatment of the mine water did not start until the late 1980s. Nonetheless, a clear recovery has taken place in the aquatic ecosystems close to Falun during the 1990s. To explain this rapid environmental improvement, it is necessary to understand the speciation and bioavailability of the toxic metals, as well as the antagonistic effects of some metals (such as Zn) towards the manifestation of harmful effects of other, more toxic metals.

4.4.1 Background and definition of the case to be discussed

The Falun Copper Mine and the Falun area in central Sweden are unique for at least two reasons:

- It was probably the most long-term continuous mining operation in the world, extracting mixed sulphidic ores (mainly containing copper, zinc and lead), for more than a millennium – in industrial scale for at least 450 years – until the operation ceased in 1992.
- From no other place in Sweden have so many and so large quantities of metals and acidifying substances been released to the environment as from the Falun Copper Mine. Thus, the region can be said to have a kind of “record” of long-standing and severe environmental damage.

Since industrial-scale exploitation of the Falun sulphidic ore started in the middle of the 16th century, the total output of metals from this ore has been about 0.4 Mt of blister copper, about 0.5 Mt of zinc, 0.16 Mt of lead, 380 t of silver and 5 t of gold. It may be mentioned that the mining operations were run by what is assumed to be the world’s oldest limited company, Stora Kopparberg Bergslag, limited at least since 1288, according to existing documentation.

The ore body has been severely weathered, especially during the glacial periods, when the rock was fragmented and spread around. Parts of the glacial till around the Falun mine therefore contain naturally high concentrations of metals and sulphur. This matrial decomposes steadily, so the groundwater and the watercourses in the neighbourhood exhibit naturally elevated levels of metals and sulphate. However, the additional – sometimes sharp – increase in these concentrations, caused by the mining operations, is the main subject of the subsequent discussion.

Over the centuries, various kinds of mining wastes, more or less rich in extractable valuables, have accumulated in the close surroundings of the Falun Mine. Among the different waste types, that can still be found in large heaps around the mine or as fill materials on which the city of Falun was built, are the rock overburden or gangue, the poorer parts of the crushed ore (called “cobbing”), tailings from the modern concentration procedures, roasted pyrite and slags. Only the slag from ore smelting has been estimated at an amount of at least 6 Mt. The weathering of all this waste material has constituted an enormous source of metal and sulphur emissions to the environment. As if this was not enough, a great cave-in occurred in the main ore body in 1687, which created a wide, gaping hole nearly 100 metres deep and beneath this, a huge amount of mineral-rich rubble down to a depth of nearly 350 m. As a result of this “Great Cave-In”, the innards of the Copper Mountain were exposed to atmospheric oxygen, which accelerated the weathering process. Rainwater percolating through the rubble carried the weathering products to the bottom of the mine, from where they were pumped to the surface and discharged into the Gruvbäcken Creek, which empties into River Faluån, Lake Tisken and Lake Runn, before the water reaches River Dalälven (cf. map in Figure 4.2).

The pumping and discharge of the heavily polluted mine water to the Gruvbäcken Creek continued until 1987, when purification of the mine water was initiated.

Figure 4.2. Map showing the main water courses in the Falun area, especially the receiving waters of the mine waste: the Gruvbäcken Creek, River Faluån, Lake Tisken and Lake Runn, which empties into the great River Dalälven. Personal commuication, Lindeström, 2002.

According to current estimates, a total of about 6 Mt of sulphur dioxide and 15 thousand tonnes of copper have been emitted to the atmosphere and between 0.5 and 1.0 Mt of copper, lead, zinc and cadmium have been discharged to the surrounding forest soils and watercourses. Therefore, the Falun area and the whole drainage basin of River Dalälven would be excellent objects for comprehensive studies of the fluxes of metals from mine waste to rivers and of the environmental impacts of metals such as copper and zinc as an input for environmental risk assessments. It would also be possible to utilise the Falun area for studies of whether, and how, nature has succeeded in adapting to often extreme environmental conditions, and to what degree nature has recovered after the emissions of pollutants have ceased.

The initiative to the ongoing “Falun Project” was taken soon after the Swedish Government appointed the “Dalälven Delegation” in 1988 and stipulated that it should devise a programme to purify River Dalälven within ten years. In the first report from this delegation, it was proposed to investigate measures for cleaning up the mining wastes, mainly in Falun and Garpenberg. Later on, in 1992, the Falun Project was launched. Its objective was to measure and reduce the metal discharges from mining wastes in Falun to an acceptable level during a time period of 15 years. The person in charge of the very comprehensive monitoring programmes in River Dalälven, including the lakes and watercourses linking the Falun Mine area with the main river (Lakes Tisken and Runn, River Faluån and the creek Gruvbäcken) has, from the beginning been Lennart Lindeström. He recently published a book giving a detailed account of "The Environmental History of the Falun Mine" (Lindeström, 2003). Some of the highlights from this book that are particularly relevant for our understanding of the consequences of large-scale and long-term metal fluxes from non-ferrous mining wastes, especially the biological effects of this extremely strong and persistent metal exposure of the aquatic ecosystems in the receiving water bodies will be briefly summarized in this sub-chapter.

4.4.2 Brief description of the level of pollution with metals in water and sediments of receiving rivers and lakes

The total loading of the watercourses within and downstream the city of Falun with metals emanating from the mine water (untreated until 1987) and the combined mining waste deposits in the Falun area is shown in Table 4.13 as concentrations of metals in the affected watercourses (rivers and lakes). Data are given from measurements prior to 1978 and as averages for the 1990s. In the 1970s, the pH of the outflowing mine water was 2.1-3.1, while pH values between 3.6 and 6.2 usually were reported from the Faluån River. During the 1990s, i.e. the period after the start of the mine water treatment unit, the maximum copper concentration in the Faluån river was 300 µg/l and in the bottom water of Lake Runn, 60 µg/l.

Average total concentrations of copper and zinc in the water of River Dalälven, upstream of Lake Runn's outflow into the river are about 0.5 µg Cu/l and about 2 µg Zn/l. As will be seen in Table 4.13, the elevation of the concentrations of copper and zinc in the lower reaches of River Faluån, compared to these natural, regional background levels, would thus be about 140 times for copper and about 1,000 times for zinc, resulting from the natural mineralisation and man's exploitation of the ore body in Falun.

The table also shows that the measures taken in the late 1980s to protect the environment, such as the treatment of the mine water starting in 1987, has resulted in a certain decrease in the average metal concentrations in lower River Faluån: a 3½-fold reduction in copper and a 2-fold reduction in zinc concentrations. However, in the relatively large Lake Runn, the water was still in the 1990s highly contaminated with copper and zinc. Also in River Dalälven, there is a clear impact of the old activities in the Falun area, e.g. shown as a 2-fold elevation in total copper concentrations and a 5-fold elevation in zinc concentrations when the river passes the Falun area (Lindeström, 2003).

Table 4.13. Metal concentrations in the mine water pumped from the Falun Copper Mine and in the receiving rivers and lakes prior to 1978 and averages for the 1990s. All concentrations are total concentrations in the water, expressed as µg/l. After Lindeström, 2003.

Sampling point	Period	Flow l/s	Copper	Lead	Zinc
Mine water	Before 1978	6.3	60×10^3	--	2×10^6
Faluån River, below	Before 1978	6×10^3	250	--	4,100
Faluån, above city	1990s	3×10^3	6.2	0.23	15
Faluån, below city	1990s	6×10^3	70	2.2	2,000
Lake Runn, surface	1990s	--	8.3	0.26	190
Lake Runn, bottom	1990s	--	14	0.36	410
River Dalälven below Lake Runn	1990s	0.32×10^6	1.2	0.22	20

In contrast to copper and zinc, the average lead concentration in River Faluån is only about 10 times higher than in surrounding streams. Thus, much less lead has been released from the mining wastes compared with the other metals. Moreover, lead is effectively immobilized in the bottom of Lake Runn, probably as an effect of co-precipitation with iron, which flocculates and settles in great amounts in the lake. Iron was one important constituent of the ore exploited in the Falun Mine. The calculated degree of immobilization of metals in Lake Runn, expressed as an average for the years 1990-97, was for copper – 45%, for lead – 80%, and for zinc – 25% (Lindeström, 1999). This means that some 75% of the annual input of zinc to Lake Runn, or 180,000 tonnes, are carried out into River Dalälven, while only 55% of the copper input (5,800 tonnes) goes the same way.

The above considerations leads to the conclusion that Lake Runn – especially its bottom sediment – acts as an effective trap of the metals released from the mine wastes in Falun. The same is true for Lake Tisken, situated just upstream of Lake Runn and being the first sedimentation basin downstream of the Falun Copper Mine. In order to give an idea about the present levels of metals in the superficial sediment layers in these two lakes, some data is summarized in Table 4.14. For comparison, the median metal concentrations in the sediment surface of 30 small lakes within the River Dalälven drainage basin are also presented.

Table 4.14. Concentrations of some metals in superficial sediments in Lakes Tisken and Runn immediately downstream of the Falun Copper Mine and median value for small lakes in the catchment of River Dalälven. Concentrations in µg/g DM. After Lindeström, 2003.

Lake	Copper	Lead	Zinc	Cadmium	Iron
Lake Tisken	3,600	2,700	11,000	19	<300,000
Lake Runn, NW	3,800	900	12,000	29	140,000
Lake Runn, central	1,400	300	7,000	13	170,000
Lakes, Dalälven basin	30	70	350	1.8	59,000

Copper concentrations in sediments in the nearby sedimentation basins are elevated above regional background levels by 120-130 times and zinc concentrations by some 30-40 times, the latter showing about the same degree of elevation as lead. Cadmium concentrations are much less elevated. As a matter of fact, the copper level in the lake sediments, just below 0.4%, is not much different from the copper grade of the ore being extracted during the last few decades of the Mine's life-time. Even when looking at the somewhat lower levels of metal contamination in the sediments of central Lake Runn, it can be noted that these levels are still 3-15 times higher in the case of copper, and 8-25 times higher in the case of zinc than the levels observed in the most contaminated superficial sediments in the inner waterways of the city of Stockholm.

4.4.3 Biological responses to the enhanced metal concentrations

One of the key questions asked by Lennart Lindeström when he set out for the compilation of all the monitoring data from the Falun area was: "Can plants and animals live in these waters under the prevailing environmental conditions ?" The question is pertinent because no other watercourse of any importance nor any other lake of the same size in Sweden exhibits such high concentrations of one or several metals as the Faluån River and Lake Runn.

Almost no plants and animals existed in the smaller Lake Tisken up to the end of the 1980s, when the mine water was first treated. The only specimens of higher life observed in the early 1980s were a few aggregations of rushes, sedges and water moss, as well as two species of chironomid midge larvae (Lindeström, 2003). However, after the mine water treatment came into operation (in 1987), new plants appeared in the lake. By 1996, large populations of several species of aquatic plants were recorded, especially the bulbous rush (*Juncus bulbosus*), but also, for example, yellow water lily and floating carpets of *Sparganium gramineum*. The mass occurrence of bulbous rush is interesting: at the turn of the millennium, this usually low-growing species with a bulbous, onion-like base, had completely taken over the lake habitat and covered the entire surface of Lake Tisken. The plant is unusually tolerant of acidic conditions and often occurs in mineralised areas, where – in the old days – ore prospectors utilized it – under the name of "ore grass" – as an indicator of the occurrence of lake ore. By the end of the 20th century, fish, such as pike, perch and roach, were also reported from Lake Tisken. The fish was much smaller than normal and had probably not been hatched in the lake, but migrated in from adjacent bodies of water.

The benthic fauna of Lake Runn was investigated in 1978, in 1982 and in 1996. On all these occasions, bottom-dwelling fauna was found, but at the first two investigations, the number of animals and their biomass were much smaller than normal for a lake of this nutritional status and size. During the 1996 survey, a total of 40 different taxa (species or genera) were found in Lake Runn. This number is somewhat lower than expected and may be due to the fact that animals are virtually absent at a depth of one metre, due to man-made fluctuation of the lake's surface. On deeper bottoms in Lake Runn, the number of individuals and the biomass were rather similar to what is found in other oligotrophic lakes in the region, see Table 4.15.

Table 4.15. Macroscopic benthic fauna in Lake Runn and similar oligotrophic lakes in the region investigated in 1996. Results are shown separately for shallow and deep bottoms (above and below the thermocline). After Lindeström, 2003.

Lake	Max. depth, m	Phosphorus (µg/l)	N:o of taxa	Individuals /m² shallow	Individuals /m² deep	Biomass, g /m² shallow	Biomass, g /m² deep
Runn	27	9	40	1,000	420	700	860
Venjan	36	10	25	270	2,700	160	6,800
Siljan	130	5	53	1,200	280	1,800	330
Skattungen	48	6	43	790	230	490	320
Rogsjön	45	3	57	2,400	760	2,600	1,400

Individual density of benthic animals and biomass per unit area below the thermocline were lower in the large Lake Siljan than in Lake Runn. In Lake Siljan, however, there are sensitive crustaceans, so-called glacial relict species, which do not exist in Lake Runn. Nor were any mussles found in Lake Runn in 1996 at depths greater than two metres. It was also noted that a large portion of the oligochaete worms were abnormally small in size. Thus, the benthic animal community in Lake Runn is undoubtedly affected by one or more factors that do not have anything to do with the general nutrient status of the lake. Most probably, the presence of high concentrations of metals is the cause of this situation (Lindeström, 2003).

The most obvious explanation is that one or several of the metals have a direct toxic action on the animals. However, it is also highly likely that the consistency of the bottom substrate (extremely "fluffy" due to the precipitates of iron) makes it impossible for many species to establish themselves on the deeper bottoms of the lake. Moreover, these iron compounds bind e.g. nutrients such as phosphorus, which would lead to a nutrient shortage for certain animals, a possible explanation to the small size of worms.

As far as the phytoplankton community is concerned, it was reported from a study in 1982 that the biomass of algae in central Lake Runn was slightly lower than could be expected in view of the nutrient status of the lake. The opposite was true during the investigations carried out in the 1990s, namely that the phytoplankton biomass in Lake Runn was higher than in other lakes in the region with equivalent nutrient status (Figure 4.3). Also to the contrary of the situation in 1982, no observations of deformations of other visible changes in algal cell morphology were made in the lake. The average number of algal species in August in the waters of Lake Runn was normal during the samplings 1990-2000, judged from the relationship between phosphorus status and number of species recorded in many lakes in the region. However, the total number of species (about 70 species) observed at any time of the year in the lake during the same period was slightly lower than expected. This has been interpreted as a possible sign of a genetical adaptation of the algal community to higher metal concentrations in Lake Runn (Lindeström, 2003).

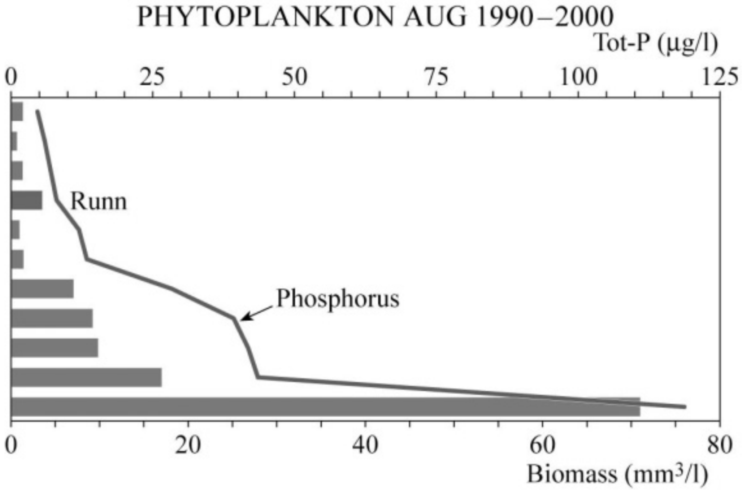


Figure 4.3. Average biomass of phytoplankton in August in several lakes in the River Dalälven catchment in the period 1990-2000 as a function of the nutrient (phosphorus) status of the lakes. Biomasses expressed in mm^3/l. Reprinted with permission from Lindeström, 2003.

The fish fauna, finally, in Lake Runn during catches made in the period 1980-2001 was found to consist of at least 12 different species: bleak, bream, burbot, crucian carp, pearch, pike, roach, rudd, ruffe, silver bream, smelt and vendace. There is presumably also at least eel in the lake, but this species in not readily caught in the nets used. The age distribution of perch was normal in the whole lake and that of roach was normal at least in the southern parts of the lake. Also the growth of perch was found to be normal as compared to other lakes in the region. Finally, physiological surveys showed that the health conditions of perch, e.g. the liver size, the blood values, etc., were normal. The only observation of a deviation from the normal status was that small roaches were underrepresented in the north-western part of the lake, near the city of Falun, as well as in the central lake. In both cases, the lower number of young roach may be due to the lack of convenient spawning areas in these parts of the lake, due to the presence of a dense carpet of fluffy iron precipitates. No deformations of individual fish have been reported from Lake Runn.

Analyses of the content of copper and zinc in the liver of perch have been conducted in many of the lakes in the River Dalälven drainage basin in 1996. The results of these analyses are presented in diagrams together with the concentrations of the same metals in the water of the lakes, see Figure 4.4. Data from Lake Runn are represented by three bars, corresponding to fish from the south (S), central (C) and north (N) parts of the lake.

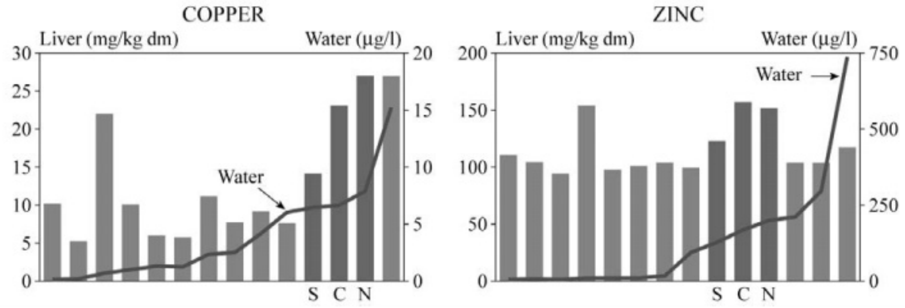


Figure 4.4. Concentrations of copper and zinc in water (curve) and in liver tissue of perch (bars) in several lakes in the River Dalälven drainage basin. Concentrations in water are expressed as µg/l and in liver tissue as µg/g DM.Reprinted with permission from Lindeström, 2003.

4.4.4 Some conclusions from the Falun studies

The great importance the Falun Mine has had for the technical, economical, social and political history of Sweden and Europe has been recognized by the designation of the Mine and parts of Falun city as a World

Heritage Site by UNESCO. But the long history of environmental impacts of the Mine is also unique and deserves similar recognition.

It appears that the soils and terrestrial ecosystems surrounding Falun have recovered to a remarkable degree during the 20th century. How has this been possible in view of the massive pollution that took place over so many centuries ?

The aquatic environment has had much less time than the terrestrial ecosystems to respond to pollution abatement measures, since the mine water did not begin to be treated until the late 1980s. Nevertheless, a recovery of the aquatic ecosystem can clearly be seen in the Faluån River catchment and its lakes during the 1990s. For example, it is surprising that the environmental conditions are not worse than they are in Lake Runn, despite the large input of metals to which the lake has been – and is still – subjected. To explain this, the speciation and the bioavailability of the metals are a central issue. Both various environmental factors and the presence of other metals certainly influence the bioavaiability and toxicity of a certain metal. Also the possibility of acquired higher tolerance of the biological communities or adaptation of populations to the elevated metal concentrations must be taken into account.

4.5 Summing up: Fluxes of Cr, Cu, Ni and Zn from Society to the Environment

The many studies of trace metal fluxes from society to the environment in various countries have provided data that made it possible to close the input-output balance for at least some important trace metals, if the calculations are based upon "total" metal (all chemical species lumped together). However, there is still very insufficient information on the actual metal species in most studied metal flows. Even less knowledge is available on the various transformations, from one set of species to another, that may take place during transport from the source to the final environmental sink for the metal. Such information is crucial for a proper assessment of the environmental risks associated with the spectrum of current metal uses in society.

In chapter 4, some critical steps in these large-scale metal fluxes have been discussed and a few important examples of recent advances in metal-flux research have been presented. These examples covered:

- *a careful quantification of environmentally induced variations in metal release following corrosion of metal surfaces in the urban environment, and a description of changes in metal speciation in the runoff liquid during transport away from the source;*
- *a demonstration of how difficult it may be to establish relationships between metal releases from a certain road traffic intensity and biological uptake and effects in the receiving aquatic environment;*
- *a survey of the sources of trace metal fluxes to municipal sewage treatment plants and from there to sludge and agricultural land, as well as a discussion about metal speciation, uptake and biological effects in sludge-amended soil ecosystem;*
- *an assessment of ecosystems exposed to heavy and extremely long-term metal pollution, caused by mining activities for more than a millennium, including a discussion of why today's impacts on biota appear to be relatively limited.*

One of the remaining central problems, when interpreting results from field studies regarding impacts of trace metals on aquatic biota and on predators (including man) consuming fish and other seafood, is how to separate the risks associated with current polluting activities from the risks caused by exposure to the cumulative mass (e.g. in bottom sediments) of a long history of metal pollution. Some recent examples from Stockholm provide a possible way of solving this problem, which is partly related to the difficulty in finding good estimates of the natural regional background concentration of trace metals in aquatic sediments.

Although some important advances have been made recently, it can still be concluded that our knowledge about the quantitative aspects of trace metal fluxes from society to the environment is insufficient and scattered. In particular, our knowledge about the exact quantities flowing from a specific source to different environmental compartments is far from complete. Furthermore, the precise composition of a certain metal flow with respect to the distribution between different metal species is usually not well described. Even less so are the various transformations from one set of species to another during the transport of metals from the source to the final environmental compartment, under the successive influence of different environmental conditions along the transportation route.

Nonetheless, it is extremely important to have at least a rough idea about the speciation and the factors governing the bioavailability of the metals released from society and their potential for causing toxic effects in terrestrial and aquatic ecosystems as well as on man.

Even if, today, we are able to close the input-output balance of the most investigated among the trace metals, taken as “total” metal (all species combined), there is very insufficient information on the flows of, for instance, the bioavailable fraction of a certain metal. Researchers in Sweden have devoted a great deal of interest to quantify the fluxes of many metals from society to the environment, and a few among them have had the ambition to separate the bioavailable fraction from the rest and to quantify that fraction. But still today, we are not in a position to draw a complete picture of the flows of even the most relevant metal species and based on that, carry out a comprehensive environmental risk assessment of metals such as chromium, copper, nickel or zinc.

One of the obvious problems involved is that most studies published in the literature are focusing on just one or a few aspects of the complex problem: for example, one study may be providing a very complete account of the sources of a certain metal and even quantify the amounts emitted per unit time, while another study contributes with a complete picture of the different metals entering into an STP and even of the distribution of the metals between sludge and effluent. However, integrating the different parts of the complex reality is still very rare and, particularly, reports describing the dynamics of the processes with the likely transformations between the most important chemical species are almost non-existing.

From what has been described in this chapter on the different approaches taken by Swedish researchers over the past few years, it can be concluded that:

- Researchers who have quantified the metal fluxes caused by corrosion and runoff from roofs and other constructions are among the very few (in Sweden) who try to make a serious description and quantification of the metal species involved, especially of the fraction that is bioavailable. However, although we have today a fairly good idea about the situation at the edge of a roof or just below a metal construction, we still need a good description of how the composition of metal species change during transport of the runoff water: How much of each species (in a city like Stockholm) goes via the combined sewer system to the STP, how much via the separate storm-water sewer, and how much would remain in the near-field environment due to chemical binding, adsorption, etc ? During this transport process, how much of the original metal species will be transformed into less (or more) mobile species ? Research is in progress in this field, and in a few years, the understanding of these processes is supposed to be considerably improved.

- To follow the metals released from road traffic related activities via storm-water flows to receiving lakes or rivers and there, to describe their uptake and possible effects on biota is an extremely difficult task, because the systems and processes involved are so very complex. It is seldom possible to separate out a certain quantity of a metal occurring in a storm-water sewer – and even less one occurring in a lake sediment – from all the rest and label it as a "traffic-generated" pollutant. Only when dealing with metals that are very strongly linked to the traffic sector, such as tungsten or lead, it would be possible to attribute the body burden of e.g. tungsten in an aquatic animal to its origin, the tyre studs of a car. For metals with a more diverse use in society, it might be almost impossible to base any environmental risk assessment of a certain traffic activity causing metal releases to the environment on biological studies in receiving waters.

- In many respects, it seems a lot easier to follow the chain of mobilised metals from the (relatively) closed system of wastewater disposal in domestic, commercial or small-industrial areas to the public sewer and the STP. From there it is relatively uncomplicated to describe the distribution of a certain metal between the sludge and the effluent from the STP to the receiving environment. As far as the sludge is concerned, the key question is, of course, whether or not the sludge would be recycled back to agricultural land and thus back to the crop-food cycle. When sorting out the relevant issues in this metal flux, it seems to be most important to focus the metal speciation efforts on the agricultural soil compartment and answer questions, such as:
    - what is the ability of the metal to be taken up by the roots of the actual crops?
    - what metal species can affect the soil micro-organisms?
    - what is the potential for the metal to be leached out to the groundwater?

- Even easier might be to describe the consequences of metal release from mining wastes, since in this case, the metals being released from the waste heaps usually are so dominant in the system under investigation that all other sources would be totally marginal. If it is then possible to conduct biological studies of both metal uptake and effects on sensitive components of the aquatic ecosystem, the results obtained, e.g. concerning established relationships between a metal

> concentration, the prevalent metal species and the biological effect, would be rather straight-forward and unambiguous. In the case of the Falun Mine, it was possible to undertake studies of all the relevant links in the chain and, even if a detailed speciation of the metals involved was not carried out in the rivers or lakes studied, the relationship between exposure and effect could be sufficiently well described to draw important conclusions. Nonetheless, there are many remaining problems to solve in relation to the Falun Mine, for example to give more comprehensive and precise explanations of the relative lack of biological effects that would be expected at the concentrations of total metal recorded.

One of the most fundamental problems of metal research conducted in the field, if the results should be used to provide a basis for decisions on necessary administrative or technical measures to protect human health and the environment, is how to differentiate between the risks associated with present-day activities and risks caused by exposure to the cumulative results of a long history of metal pollution. In fact, this is an aspect that has not been given sufficient attention, but which is crucial for the correct understanding of the impacts of today's activities. In particular when conclusions are drawn on the basis of findings from sediments or sediment-dwelling organisms, i.e. when information is based on the natural archive accumulating the remains of many years of metal emissions, it is usually very difficult to separate the traces of the past from those of the present.

A relatively recent example from Stockholm can show how difficult it might be for the city authorities to arrive at the correct conclusions on whether or not there is a need for urgent measures to limit the use of certain metals, when the research report does not, to a sufficient extent, differentiate between effects of the past and effects of the present.

In the final report from a sub-project, being a part of the environmental monitoring in Stockholm's inner waterways, Broman et al. (2001) gave an account of the amounts of metals and organic pollutants that are deposited in "fresh" sediments. Fresh sediments were collected in sediment traps, which were kept at a water depth of 15-20 m for about one year, during each of the years 1996-97, 1997-98 and 1998-99. The traps were fixed at three different sites selected to illustrate the impact of the city on the current pollution load. One site was upstream of central Stockholm, Klubben, one in Lake Mälaren (Riddarfjärden) in central Stockholm, and the last one in Saltsjön (Kastellholmen), the brackish-water area just downstream of the central city. The authors specified that it had previously been demonstrated that in the sediment traps used, a certain fraction of the collected sediments originates from resuspended bottom sediments. At the two sites in Lake Mälaren, about 50% of the collected sediments can be estimated to be resuspended bottom sediments, while at Kastellholmen, this fraction would be 75%. However, in the evaluation of the data and presentation of the conclusions, no mentioning was made of how this difference would affect the results.

In order to illustrate the risk of misinterpreting this kind of data, we are presenting an overview of the most relevant results from the sediment traps and from analyses of the superficial sediment at the same sites, made by other researchers (Table 4.16). In the table, only the results for the two metals copper and zinc are presented. One reason for not displaying the data on chromium and nickel in this table is that no clear difference was found between the concentrations of these metals in the material collected in the sediment traps and in the superficial bottom sediments, which probably is due to the very small emissions of these metals from the anthroposphere in Stockholm.

The data in Table 4.16 regarding metals in sediment traps is averages based on four samples, (three consecutive years of sediment collection by Broman et al., 2001, and one sample reported by Lithner et al., 2003). Metals in superficial bottom sediments are averages from three samples at each site, two reported by Östlund et al., 1998, and one by Lithner et al., 2003). Since the copper and zinc concentrations in the trapped sediments were always much lower than in the sediments from the bed, it may be concluded that freshly sedimenting material is less contaminated with these metals. The question is how much less. Since the material in the sediment traps is reported to be a mixture of freshly sedimenting material and resuspended bottom sediments, the new material must be much lower in metal content than what is reflected in the sediment traps.

In Table 4.16, a plausible concentration of copper and zinc has been given, based on calculations assuming that 50% resuspended material was found at the two sites in Lake Mälaren, while 75% old material occurred in the sediment trap in Saltsjön. For comparative purposes, copper and zinc levels in zebra mussels exposed for six weeks at the same sites are also presented in the table and so are the regional background concentrations in sediments, estimated by Landner (section 5.7 of this report), and by Lithner et al., 2003, both as “unaffected superficial sediments upstream Stockholm” and as “preindustrial sediments from the Strängnäs area”. Finally, the “background” levels in superficial, oxidised sediments in the central part of the Baltic Sea are also given as a reference.

Table 4.16. Overview of copper and zinc concentrations in sediments collected in sediment traps and in superficial bottom sediment from three sites in the inner waterways of Stockholm. The table also displays calculated concentrations of copper and zinc in freshly settling material. For comparison, estimated regional background concentrations in sediments and copper and zinc levels in transplanted zebra mussels are given. All concentrations expressed as µg/g DM. After Broman et al., 2001; Östlund et al., 1998; Lithner et al., 2003.

	Lake Mälaren				Saltsjön	
Matrix	Klubben		Riddarfjärden		Kastellholmen	
	Cu	Zn	Cu	Zn	Cu	Zn
Sediment traps (4)*	100	280	140	430	320	650
Surf. bottom sedim, (3)*	170	380	220	560	370	700
Difference	70	100	80	130	50	50
Freshly settling material (calculated)	~30	~180	~60	~290	~140	~490
Background (Landner) (L. Mälaren)	37	120				
Background (Lithner)						
- recent sediments	50	245				
- pre-industrial level	35	130				
Central Baltic Sea - oxidised surf. sediment					45	190
Zebra-mussels (sect. 4.2.3)	11	180	12	320	10	240

* number of samples

Due to the geological background in the Lake Mälaren catchment, the pre-industrial background of copper in sediments is relatively high (35-37 µg/g DM) as compared with the median value in pre-industrial sediment layers in forest lakes (about 15 µg/g DM).

The table also shows that concentrations of copper and zinc in surficial bottom sediments are higher than in the material collected in the sediment traps. The difference was greatest at the site in Riddarfjärden in central Stockholm. Considering that the traps in Lake Mälaren did contain about 50% resuspended bottom sediments, the freshly settling material would have a copper concentration close to the regional background level (35-50 µg/g DM) at the site Klubben, and about twice as much at the site Riddarfjärden. However, at Kastellholmen, the freshly settling material appears to be clearly contaminated by anthropogenic copper. A similar picture emerges for zinc: no or very low contamination at Klubben, a certain impact in Riddarfjärden, and a clear enrichment, probably caused by anthropogenic zinc at Kastell-holmen.

In a follow-up study in May and June 2002, IVL carried out a comprehensive sampling of bottom sediments from water-ways and lakes in Stockholm and in the adjacent Svealand coastal region, in order to determine the concentrations of 32 Water Framework Directive (WFD) priority substances (Sternbeck et al., 2003). Among the analysed substances were 10 metals, including Cr, Cu, Ni and Zn. The sediment samples were taken from the top (0-2 cm) layer and each sample was a mixture from at least 8 sediment cores. Samples were digested with concentrated nitric acid in the autoclave before chemical analysis. Thus, no attempt was made to separate different chemical species, such as the bioavailable fraction.

When the trace metal levels in the sediments sampled in central Stockholm were compared with preindustrial levels, or upstream areas, a slight enrichment was noted for Cu and Zn. But when levels in central Stockholm were compared with those in small lakes in the region, no enrichment was demonstrated, except for a small enhancement of Cr. In the coastal region, just downstream of Stockholm, there was a fairly low impact of trace metals, with Cr and Ni being almost identical to the preindustrial levels, while Cu and Zn were slightly elevated in the archipelago of Stockholm, but levels were lower than in surface sediments from the open Baltic proper (Sternbeck et al., 2003).

The actual metal concentrations in central Stockholm sediments were also compared with the levels found in 1997, and it turned out that there was a general decline in the concentrations of Cu and Zn, while those of Cr and Ni did not show any change. The decreasing trend for sediment metals over the last 5 years was found to be in agreement with general long-term trends in the region (Sternbeck et al., 2003).

5 SPECIATION, MOBILITY AND BIOAVAILABILITY OF METALS IN THE ENVIRONMENT

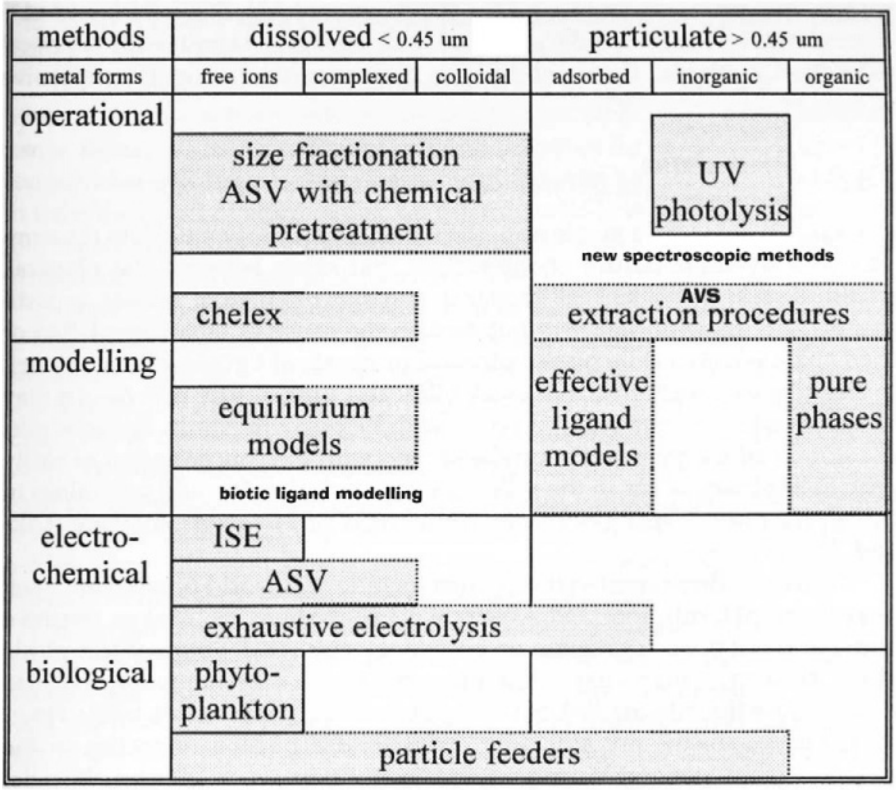
5.1 Introduction

We know today that trace metals in sediment and water occur not only in one single chemical form, but at the same time in a variety of physicochemical associations (species). We also know that these forms differ in their mobility and bioavailability. Plenty of analytical techniques have been developed to measure and characterize these metal forms. Assessing fate and biological effects of metals in the environment based solely on total concentrations is no longer state-of-the-art or scientifically motivated. In fact, a vast amount of knowledge has accumulated in the scientific literature during the last 3 decades, especially for those metals (Cr, Cu, Ni, Zn) that were selected in this study, that clearly illustrates the decisive role of metal speciation when metal bioavailability and toxicity in the environment have to be assessed. Although most water or sediment quality criteria for metals are still based on the total concentration of the metal in question, it is becoming more and more evident that also the regulatory society is increasingly considering speciation when environmentally relevant metals are monitored and assessed.

5.1.1 General considerations

During the last three decades, evidence has become overwhelming that it is the particular chemical form or association (species) of a metal and not its total concentration, that controls its *geochemical* (mobility, reactivitiy) and *biological behaviour* (bioavailability, toxicity) in natural, contaminated, or man-made systems. This growing insight has inspired the development and application of new sophisticated chemical methods, including advanced sampling and instrumental analytical techniques, modelling approaches, designed to tackle questions regarding speciation and bioavailability of biologically relevant trace metals. Table 5.1 provides a more generalized overview about current main speciation techniques.

Table 5.1. Overview of currently used speciation approaches (modified after Turner and Whitfield, 1982)



methods	dissolved < 0.45 um			particulate > 0.45 um		
metal forms	free ions	complexed	colloidal	adsorbed	inorganic	organic
operational	size fractionation ASV with chemical pretreatment				UV photolysis	
				new spectroscopic methods		
	chelex			AVS extraction procedures		
modelling	equilibrium models			effective ligand models		pure phases
	biotic ligand modelling					
electro-chemical	ISE					
	ASV					
	exhaustive electrolysis					
biological	phyto-plankton					
	particle feeders					

Metals in water, sediment and soil occur both as dissolved, colloidal and particulate species depending on the particular environmental conditions. *Dissolved* metal species include the free unhydrated or hydrated metal ion (M^{n+}), as well as dissolved organic and inorganic complexes. *Colloidally* bound metals are mainly associated with amorphous Fe and Mn oxyhydroxides, or humic substances. *Particulate* metal forms can occur as discrete chemical forms (precipitates), adsorbed onto solid surfaces of hydrous oxides and organic substances, or as intermediates (coprecipitates) (see Reuther, 1999).

Despite obvious progress in developing a variety of laboratory-based speciation techniques, suitable *in-situ field methods* are still not existing to analyse the original species composition and availability of a metal for chemical and biological reactions in a distinct sample matrix (see Hirner et al., 2000, and Adriano, 2001). Main reasons why these techniques are still hardly used for official monitoring and regulatory purposes, and why most controlling bodies still seem to ignore the obvious advantages of metal speciation over just determining total concentrations, are the existing deficiencies in the selectivity of methods, in analytical precision, reproducibility and accuracy, and not least the high equipment costs involved. In the following, we will show that any serious attempt to assess the risk associated with the occurrence of trace metals will nevertheless need site-specific data about the true chemical form, in which a particular metal exists.

5.1.2 Definitions

When examining current definitions used in the relevant literature, the concept of *chemical speciation* seems to mean both (1) the analysis of typical chemical species (i. e. free ionic, complexed, adsorbed, precipitated or co-precipiated forms), and (2) the partitioning of metals into these forms and their transformation in environmental systems (Reuther, 1999). At the same time, speciation has become a rather holistic attempt to integrate both chemical (mobility, reactivity) and biological (bioavailability, toxicity) aspects of transport, uptake and accumulation processes (see Tessier and Turner, 1996). However, an obvious confusion can be observed in the scientific literature regarding the use of the term "*bioavailability*". Metal bioavailability is a concept, which is still hard to define. It may include 1) the physico-chemical availability of metals in the exposure medium, 2) the actual demand of biota, and 3) the toxicological behaviour of metals inside the organisms' body. Chemical concepts for defining bioavailability seem further developed than biological ones. When speaking about "bioavailable metals", Lee et al. (2000a), for example, address metals that are available from environmental media for bioaccumulation, whereas "bioaccumulation" in turn is supposed to be an indicator of a particular metal dose. Also Brun et al. (2001) treat "bioavailability" more quantitatively, namely as the portion of the total metal concentration (here: copper) in soil that can be taken up by the roots of a given plant (in contrast to uptake, toxicity and accumulation). Ruby et al. (1999) distinguish between *absolute* (the fraction reaching the target compartment) and *relative* (or comparative) bioavailability (of different forms or for different exposure media containing the substance).

They also use the term "*bioaccessibility*" when it comes to the fraction that is soluble in the gastrointestinal tract and consequently available for direct internal absorption in higher animals. A more restricted meaning of "bioavailability" is given in Morton et al. (2000), who associate it with the amount of a metal that can be taken up by microorganisms such that a physiological response is observable. In a similar way, Allen (2002) adopted to the definition that bioavailability ”is the amount or concentration of a chemical that can be absorbed by an organism thereby creating the potential for toxicity or the necessary concentration for survival”. Bioavailability should be considered as a dynamic process consisting in a physicochemically driven desorption (the *chemical supply or availability*) and a physiologically driven uptake process (*biological availability*). Because the level of biologocally available metals must reach a certain threshold level at the target site before effects can occur (body residue concept), *toxicological bioavailability* was defined as the final determinant of toxicity (Peijnenburg, 2002) (see also chapter 6).

Although we know that bioavailable metals may not be also toxic, the terms “*sediment bioavailability*” or “*sediment toxicity*” are still treated in a rather indiscriminate way in the evaluated literature. The same holds for possible linkages between *bioaccumulation* and *toxicity*, which are difficult to establish, because biota can detoxify bioaccumulated metals (e. g. via metallothionein or granule induction) or even develop tolerance to accumulated metals. Moreover, there is an emerging evidence that metals bioaccumulated from food have novel chronic adverse effects on aquatic biota (see chapter 7, and references given in Lee et al., 2000a).

Here, we will use the term “bioavailability” to designate those metals or metal species that can be taken up by organisms both in an *active* (e. g. by extracellular digestion) or *passive* (e. g. by diffusion across cell membranes) way.

5.1.3 Fundamental properties of the selected metals

Metals in natural waters rather seldom occur as belonging to one of several well defined, distinct categories, e.g. dissolved, colloidal or particulate phases. They rather occur as *intermediates* between or *mixtures* of these categories, depending on a variety of physical, chemical and biological factors. But in most cases, only total concentrations are still measured, which means the sum of all these differently acting phases. Moreover, one should keep in mind that every measurement in the field or laboratory is just a *snapshot* of the dynamic reality, governing the complex interaction between metals and the environment.

Just to illustrate the difficulties met by even leading scientists today in their attempt to increasingly consider speciation aspects in monitoring and assessing the impact of metals, the “1995 Nordic Lake Survey”, a remarkably comprehensive field survey including Norway, Sweden and

Finland may be mentioned here (Lydersen et al. 2002). One main objective of this study was to investigate the distribution of trace metals in lakes in relation to environmental factors, like DOC and pH (Lydersen and Löfgren 2002). Although lacking own speciation data, the authors succeeded to demonstrate that a sound evaluation and interpretation of the bulk analytical results without knowing the particular speciation of the selected trace elements (Zn, Cd, Cu, Pb, Ni, Cr, As, Al) is hardly possible. When discussing the outcome in the light of what we already know on metal speciation, the authors emphasize that their conclusions and the implications of the generated data should be treated with great care, due to the lack of own chemical speciation data. As an example, the conclusion that *remobilization* of metals in limed lakes, after these systems may re-acidify, may be insignificant, was only supported by data on total metal concentrations, and has to be seen for this reason with some reservation. Also the reasoning that possible ecological risks associated with **Cr, Cu**, Fe, Mn and **Zn** seem to be very low and may be only in those exceptional cases, where water concentrations are above the *lowest biological risk* (LBRL), was based on the use of measured total concentrations (see Table 5.2).

Table 5.2. Percentage of Swedish and Norwegian lakes below the lowest biological risk levels given as total concentrations in µg/l according to Norwegian and Swedish criteria (from Lydersen et al., 2002)

			%			
Element	country	LBRL[a] µg/l	Norway non-limed	Sweden non-limed	Sweden limed	Finland non-limed
Zn	Norway	50	99.8	99.6	99.6	99.8
	Sweden	20	99.3	99.3	98.9	99.4
Cd	Norway	0.2	99.5	98.6	98.9	99.8
	Sweden	0.1	96.6	95.5	97.7	99.4
Cu	Norway	3.0	99.3	98.4	99.6	99.1
	Sweden	3.0	99.3	98.4	99.6	99.1
Pb	Norway	2.5	98.4	98.2	98.1	99.8
	Sweden	1.0	91.5	93.0	93.5	98.3
Ni	Norway	5	99.8	99.6	99.6	98.9
	Sweden	15	100	99.8	100	99.4
Cr	Norway	10	100	99.8	100	100
	Sweden	5	99.9	99.6	100	100
As	Norway					
	Sweden	5	99.8	99.8	100	100

[a]LBRL=lowest biological risk level

In this context, we have to be aware that assessing the environmental risk of water-borne or sediment-associated trace metals without considering their chemical speciation is today no longer state-of-the-art and may ultimately lead to wrong assumptions, decisions and predictions concerning the fate and effect of potentially toxic metals.

Just to call back to mind the complexity of interactions between metals, site characteristics and biota controlling the behaviour (speciation, bioavailability and toxicity) of metals in natural systems (soils, sediments and waters), a short overview will be given here of what we already know in this context from field studies for the metals Cr, Cu, Ni, and Zn, which have been selected for this evaluation and update. The information was mainly taken from Lydersen et al. (2002).

Chromium

Cr is a rather abundant element in nature, with *two main chemical forms*, i. e. Cr(III) and Cr(VI). Cr(III) is essential for mammals and included into the glucose, lipid and protein metabolism, in contrast to Cr(VI), which is known to be toxic to organisms. In natural surface waters, average Cr levels are around 1-2 µg/l. Scandinavian lake waters are characterized by rather low Cr levels, mainly < 1 µg/l. The risk level for biological effects in sensitive Swedish waters and for salmonids in Norwegian freshwaters was given to be 5 µg/l. Swedish and Norwegian low pH waters (pH<5.4) exhibit lower Cr concentrations (median value of 0.069 µg/l) than higher pH waters (with 0.076 and 0.087 µg/l at pH 5.4-6.0 and pH > 6.0, respectively). Also the observed high affinity of Cr to organic matter results in lower levels in Swedish and Norwegian low TOC (**T**otal **O**rganic **C**arbon) lakes (median Cr value of 0.054 µg/l at TOC < 5 mg/l) when compared to medium TOC (median Cr value of 0.14 µg/l, at TOC between 5 and 10 mg/l) and high TOC lakes (median value of 0.27 µg/l at TOC ≥ 10 mg/l).

It is supposed that soil-derived Cr is mainly transported as metal-humus complex to surface waters, one reason why humic surface waters generally show elevated Cr levels. In natural waters, Cr occurs mainly as *trivalent* (Cr^{3+}) and *hexavalent* (Cr^{6+}) chemical form. However in freshwaters, Cr^{6+} can only exist at low organic matter, Fe^{2+} and S^{2-} contents due to its rapid reduction to Cr^{3+}. In contrast, Cr^{6+} is the prevalent Cr form in seawater. In low organic waters, trivalent Cr occurs as the free metal ion (Cr^{3+}) at pH < 5.5, as $Cr(OH)^{2+}$ at pH < 6.5, or $Cr(OH)_2^+$ at pH > 4.5. Cr(III) hydroxides start to precipitate at pH ≈ 5.5-6.0. Cr(III) forms stable complexes with both organic and inorganic ligands, why it is very unlikely that it may occur as uncomplexed free ion when anionic or particulate compounds are present (see Table 5.3 below). Clay particles, humus colloids and Fe and Mn oxyhydroxides all coprecipitate Cr into lake sediments. Cr(III) is supposed to be not mobile in sediments neither at oxic nor anoxic conditions, mostly due to the formation of stable inorganic and organic complexes. In soils, Cr levels remain in general below 100 mg/kg (dw). A recent investigation of 360 mor layer samples of Swedish forest soils (cited in Lydersen et al. 2002) showed concentrations between 0.7-22 mg/kg dry weight (dw). Like for other trace metals, soil Cr concentrations can increase in the vicinity of large point sources such as iron and steel mills. Also in soils, Cr forms stable organic complexes, which may move from the A soil horizon and precipitate/accumulate together with Fe and Al compounds in the upper part of the B horizon.

Copper

Cu is a relatively common trace element in nature and the 28th most frequently found in the earth crust. It shows often elevated surface water levels in agricultural areas (see section 5.5.7). Data obtained between 1980 and 1992 indicate for Scandinavian surface waters (lakes) a total or dissolved median concentration below 1 µg/l, whereas > 97% of the lakes had less than 3 µg/l, which is, using the LBRL levels used in Lydersen et al. (2002), supposed to be the threshold value for biological effects in sensitive waters. Cu concentrations in 126 uncontaminated groundwater aquifers in Sweden sampled in 1985 to 1987 varied between 0.05 and 9 µg/l. High TOC lakes (> 10 mg/l) show the highest (median) Cu concentrations (0.56 µg/l) in contrast to low TOC lakes (< 5 mg/l) with median concentrations of 0.31 µg/l. pH seems not to be so relevant in determining Cu concentrations in Scandinavian lake waters. Cu concentrations in soils generally range from 10 to 100 mg/kg dw depending on the mother material. A survey of Swedish forest soils with 360 samples from a mor layer (see Lydersen et al. 2002) showed median Cu concentrations in the range 3 to 36 mg/kg dw, whereas Cu in Norwegian soils is about 10 mg/kg dw in the humus layer. In sediments, Cu is efficiently retained from uptake by organisms due to the formation of stable complexes with negatively charged organic compounds. While Cu adsorption to Fe and Mn oxides and oxyhydroxides, and to organic matter, may be an efficient retention process in oxidic sediments, precipitation of Cu sulphide may constitute a major sink in anoxic sediments. Based on sediment analyses from relatively uncontaminated lakes, sediment Cu concentrations in Sweden are supposed to vary between 16 to 38 mg/kg dw (south), 10 to 34 mg/kg dw (central) and 7 to 27 mg/kg dw (north). In other European countries the measured or estimated background copper concentrations in freshwater sediments range between about 2 and 60 mg/kg dw.

Metallic copper Cu^0 is chemically rather inert and dissolves very slowly in oxic and slightly acidic environments. The *divalent form* Cu^{2+} is the normal valence state in oxic, aqueous environments and many of the divalent Cu compounds, such as Cu sulfate, are readily soluble in water. However, cupper oxide (pK = 20.5) and copper pyrite (pK = 97.6) are very insoluble in water. The Cu^{2+} ion forms *strong complexes* with hydroxide and organic ligands, e. g. humic and fulvic acids. Various biomolecules, such as sulfur-containing amino acids and proteins (e. g. metallothionein), can form extremely strong Cu(II) complexes and are used by organisms to regulate their internal copper level. Cu^{2+} is also readily adsorbed by clay particles.

Most of the different selectivity observed for metals to form complexes with ligands might be explained by ionic charcteristics such as polarizability, hydration conditions, and the ability to form stable organo-metallic complexes. However, Cu^{2+} is an exception from this rule. According to the polarizability and hydratisation energy, the degree of selectivity to organic complexes for divalents metals should be as follows: Pb > Cd > Co > Zn ~ Ni >Cu. However, Cu exhibits the strongest assymetry in coordinate positions around the Cu^{2+} ion, meaning that two ligands have larger binding lengths (Phillips and Williams, 1996; cited in Lydersen et al. 2002). Thus H_2O molecules associated in these positions are far easier to remove, explaining the strong binding between Cu and organic ligands. As a consequence, the order of bonding strength between metals becomes reversed. Based on a review of factors that are most essential for bonding strength, the usual divalent cations may be ranked as follows: Hg = Pb > Cu >> Cd > Ni > Co > Zn > Mn > Ca > Mg. Focusing on the metals that are actual for this update, Cu turns out to have the strongest association to organic compounds. Metals with a medium bonding strength to organics include Ni and Zn, while Ca and Mg exhibit the weakest bonding strength.

pK_1 values for water- or soil-derived fulvic acids have been reported to be in the range 5.5 – 7.0 and pK_2 values in the range 3.8 – 5.4 (Borg and Johansson, 1989; cited in Lydersen et al. 2002), which agrees well with values found for Cu organic complexes in water of 10 lakes in northern Sweden (pK_1 = 5.4 – 6.4; pK_2 = 4.2 – 5.0). The potential Cu binding capacity of the organic compounds in these waters was in the range 64 – 2,200 µg Cu/l (Borg and Johansson, 1989), which is extremely high in comparison with total concentrations of copper found in Scandinavian freshwaters (Lyderssen et al., 2002).

Nickel

Nickel is *essential* for plants, bacteria, humans and other mammals, but *toxic* at higher concentrations to most plants and fungi, moderately toxic to mammals and may induce allergic effects in humans. Certain Ni compounds and maybe also metallic Ni are human carcinogens. Like Cu, also Ni shows elevated surface water levels found in the surrounding agricultural areas. Scandinavian field surveys indicate that both acidity and humic substances influence its distribution in these types of waters. Most often aqueous Ni seems positively correlated with the amount of humus (TOC) substances but negatively correlated with water pH. The positive corrrelation observed between Ni and pH in a Swedish lake survey may be due to the high pH and Ni concentrations found in agricultural areas. Data collected between 1980 and 1995 in Finish, Norwegian and Swedish surface waters show a median Ni concentration of < 0.5 µg/l (see Lydersen et al., 2002). The median concentration found in Swedish and Norwegian low TOC lakes was 0.21 µg/l, whereas in medium and high TOC lakes, median values were found to be 0.41 and 0.58 µg/l, respectively. Although the obtained field data provided no significant correlation between pH and Ni in these waters, the median concentration of Ni in low pH lakes (0.22 µg/l) was somewhat different from medium to high pH waters (0.20 – 0.33 µg/l).

The global mean soil concentration of Ni is estimated to be 35-40 mg/kg dw. In the soil profile, Ni tends to follow iron and humus and can accumulate in the B horizon and in the mor layer. Also clay minerals are marked accumulators of Ni. The solubility of Ni increases rapidly if pH drops down to 4.0-4.5 in the B horizon. In uncontaminated river sediments, Ni concentrations vary in general between 1 and 150 mg/kg dw, but can reach up to 1000 mg/kg dw in the vicinity of nickeliferous deposits. Concentration levels in surface lake sediments from an unpolluted area in central and northern Sweden were in the range of 3.6 to 21 mg/kg dw. As in soils, Ni is mainly associated with hydrous oxides of Fe and Mn, with clay minerals and organic matter in aquatic sediments.

Concerning its chemical speciation, Ni occurs predominantly in the *divalent form* Ni(II), which forms *strong complexes* with organic ligands, like carboxylates, fulvates and humates. Ni forms soluble salts of chloride, sulfate and nitrate, whereas Ni oxide is only soluble under acid conditions, in contrast to Ni hydroxides, sulphides, arsenides, arsenates, and silicates, which are almost insoluble. The stability of metal complexes with humic acids decreases in the order: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+}$. Due to the chemical similarity between Ni^{2+} and Fe^{2+} and Co^{2+}, Ni can substitute these metals in a number of compounds.

Zinc

Although Zn is known as an *ubiquiteous element* in nature, it is supposed that about 96% of its release into the global environment is the result of anthropogenic activities, like electroplating, smelting and ore processing, corrosion from alloys and galvanized surfaces as well as erosion from agricultural land (see chapters 2-3 before). It is supposed that dissolved Zn in Canadian and Scandinavian freshwaters rarely exceeds 40 µg/l. High concentrations are likely to occur in acid surface waters as a consequence of decreasing pH (acid rain) and increased atmospheric inputs. In Sweden a significant negative relationship between Zn and pH has been reported, but not as evident as in northern Sweden watercourses with about half the concentration level found in southern surface waters for the same pH. From a large monitoring campaign in 1990 including > 2000 samples from 23 large rivers and 13 small streams, highest concentrations were found to be about 60 µg/l (for pH values between 4.0-7.5). Highest Zn levels reported from the 'Nordic Lake survey' in 1995 were 55 µg/l in Finland, 427 µg/l in nonlimed Swedish lakes, 56 µg/l in limed Swedish lakes, and 139 µg/l in Norwegian lakes (see Lydersen et al., 2002). But almost 90% of all lakes investigated had concentrations < 5 µg Zn/l. Beside low pH, also SO_4 can significantly increase Zn solubility and mobility in surface waters by forming soluble $ZnSO_4$ complexes. To what extent the reduced atmospheric SO_4-deposition to Scandinavian surface waters may contribute to an increasing Zn mobility remains to be answered. There are hardly reports on a significant relationship between Zn concentrations and TOC/DOC, although the data from the 1995 Nordic Lake survey seems to indicate a weak tendency of increasing Zn concentrations with increasing TOC.

Based on the 1995 survey, median concentrations of Zn in Norwegian, Swedish and Finish lakes are 1.54, 1.27, and 1.80 µg/l, respectively. Zn concentrations analysed in 360 samples from the mor layers of Swedish forest soils varied between 14 to 149 mg/kg dw (with a median value of 55 mg/kg dw). A similar survey in Norway showed a large influence of long-range atmospheric transport on Zn distribution in soils with an average value around 30 mg/kg dw in the humus layer. As for the other metals, moss analysis in Scandinavia in 1995 showed elevated concentrations in the vicinity of large point sources, such as the metal smelter in Rönnskär (Sweden) and Odda (Norway). Median concentration of Zn in Swedish groundwater is 10 µg/l, which is higher than for running (4.1 µg/l) and lake waters (1.0 in northern and 2.2 µg/l in southern Sweden).

Background concentrations are supposed to be 3 µg/l (pH > 6), and vary greatly with pH, with higher levels at lower pH. Most Zn entering surface waters precipitates into the sediment. Although Zn seems to be more associated with easily reducible (exchangeable and Mn oxide bound) and reducible sediment fractions (Fe oxide bound), in acidic (pH 5.0-5.6) lakes, more Zn seems also associated with organic fractions (likely coprecipitated with Fe-humic compounds). Sediment-bound Zn in relatively unpolluted lakes in southern, central and northern Sweden varied between 130-140, 130-380 and 59-330 mg/kg dw, respectively. Background concentrations for zinc in freshwater sediments in other European countries usually range between 12 and 150 mg/kg dw. Concerning aqueous Zn speciation, the free Zn ion coordinates with water molecules to form the octahedral $Zn(H_2O)_6^{2+}$ in the absence of inorganic and organic ligands. At pH 4-7, Zn exists in freshwaters almost completely as the aqua ion, while at pH 6 the free ion and $ZnSO_4$ are the dominant forms. It is supposed that about 90% of dissolved inorganic Zn occurs as free ion in addition to $ZnHCO_3^+$, $ZnCO_3$ and $ZnSO_4$. In general, Zn is increasingly released from sediments under high dissolved oxygen, low salinity and pH.

5.2 In surface waters

Advances in analysing ultra-low metal levels in dissolved form in natural waters have also favoured the development of new speciation methods designed to distinguish between complexed and free metal ions. This led to an improved understanding of why metals are bioavailable, how they affect biota and how biota, in turn, respond to metal speciation. The obtained higher precision indicated that metals in natural waters may affect biota at levels much lower than previously assumed. In general, the thermodynamic stability of metal complexes, as defined by their stability constants, is considered to be critical for metal bioavailability. New hyphenated analytical techniques could show that also kinetically stable metal complexes can form even under thermodynamically unstable conditions. That the content of free metal ions is rather low in natural waters, is supposed to be due to the formation of kinetically stable organic and inorganic complexes. Recent studies show that it is the rate of exchange of a ligand between the dissolved free metal ion and a particular complex, which determines if a metal complex is stable or not. In fact, it was recently shown that stable metal-sulphide complexes, characterized by strong binding constants (preventing them from oxidation and dissociation), may be persistent in oxic surface waters.

Although the free or hydrated metal ion is still assumed as the most bioavailable form, new research confirms that also labile metal complexes (inorganic and weak organo-metal complexes with stability constants < $10^5 M^{-1}$) may contribute to the supply of metal ions to the cell surface, depending on the rate of the flux-determining step (i. e. diffusion/transport through the supply medium, or uptake at the cell surface).

A state-of-the-art description of adsorption phenomena between dissolved metals and particulates is today based on aqueous speciation and surface adsorption models. They generate distribution coefficients (k_d-values), and include the concept of additivity of discrete surface sites in binding free and/or complexed metals to solid surfaces. New results emphasize that multiple solid phases must be taken into account when modelling metal uptake processes, and that surface and solution complexation equilibria can explain much of the observed variations for k_d in natural surface waters.

The development of highly sensitive and element-specific detectors, like electrothermal vaporization atomic absorption and inductively coupled plasma atomic emission, mass spectroscopic instrumentation, and of electrochemical methods, like ASV, and hence the tremendous lowering of the analytical detection limit (down to ppt levels) for total concentrations of almost all metals, has concomitantly supported developments to further improve the selectivity and reproducibility of chemical and instrumental separation methods (like exchange resin, dialysis membrane, competitive chelation or chromatographic techniques) to identify and quantify particular metal species in complex natural waters (see Reuther 1999 and Allen 2002). As a first approach the distribution of metal compounds in aqueous phases can be defined according to their size as dissolved (< 1 nm), colloidal (1 nm – 0.2 µm) and particulate metals (> 0.2 µm). Resulting *partitioning or distribution coefficients* (k_d) provide an estimate of the binding affinities and dynamics prevailing between metals and key water components and factors.

Recently, Town and Filella (2000) compiled the available data on the complexation (e. g. conditional stability constants, effective ligand concentrations) of some trace metals (**Cu, Zn**, Pb, Cd) in sea, estuarine and freshwaters that have been published during the past 25 years, providing a comprehensive resource and reference book for scientists, who work in the field of dissolved metal ion speciation in natural waters.

To illustrate the situation ruling the partitioning and complexation of metals in natural waters, relevant speciation and surface reactions have been depicted in Table 5.3 for aqueous chromium, as an example.

Table 5.3. Aqueous and surface complexation reactions relevant for chromium speciation (from Nikoloaidis et al., 1999)

Reaction	log K	reference*
$H_2O + OH^- \leftrightarrow H^+$	14.00	Zachara et al., 1989
$H^+ + CrO_4^{2-} \leftrightarrow HCrO_4$	6.51	“
$2H^+ + CrO_4^{2-} \leftrightarrow H_2CrO_4$	5.56	“
$Na^+ + CrO_4^{2-} \leftrightarrow NaCrO_4$	0.70	“
$Cr^{3+} + OH^- \leftrightarrow CrOH^{2+}$	-10.0	Morel and Hering, 1993
$Cr^{3+} + 2OH^- \leftrightarrow Cr(OH)_2^+$	-18.3	“
$Cr^{3+} + 3OH^- \leftrightarrow Cr(OH)_3$	-24.0	“
$Cr^{3+} + 4OH^- \leftrightarrow Cr(OH)_4$-	-28.6	“
$Cr^{3+} + Org \leftrightarrow Cr^{3+}\text{-}Org$	15.0	calibrated
$\equiv ROH \leftrightarrow \equiv RO^- + H^+$	-4.5	soil titration
$\equiv ROH + Cr^{3+} \leftrightarrow \equiv ROH\text{-}Cr^{3+} + 2H^+$	-0.1	calibrated
$\equiv FeOH + H^+ \leftrightarrow \equiv FeOH_2^+$	4.2	Zachara et al., 1989
$\equiv FeOH \leftrightarrow \equiv FeO^- + H^+$	-10.8	“
$\equiv FeOH + Cr^{3+} \leftrightarrow FeOCrOH^+ + 2H^+$	2.26	Dzombak and Morel, 1990
$\equiv FeOH + H^+ + CrO_4^{2-} \leftrightarrow (\equiv FeOH_2^+\text{-}CrO_4^{2-})$	9.8	Zachara et al., 1989
$\equiv FeOH + 2H^+ CrO_4^{2-} \leftrightarrow (\equiv FeOH_2^+\text{-}HCrO_4^{2-})^0$	19.4	“

* references given in Nikoloaidis et al. (1999)

5.2.1 Metal speciation in the aqueous (dissolved) phase

Early research in ocean waters indicated similarities in the behaviour of trace metals and nutrients vis-à-vis exposed organisms suggesting that it is not only so that *essential trace elements* may affect phytoplankton, but that phytoplankton in turn may regulate trace metal composition in the surrounding water for its own benefit (Hunter et al. 1997). These insights have been mainly possible by the development of analytical (in particular electrochemical) techniques that were able to measure *free metal ions* and metals complexed by *naturally occuring ligands of biotic origin* in ocean waters at the sub-nmol/l levels (see chapter 6). From these speciation studies it became soon clear that the so-called *free metal ion activitiy* of many essential and toxic metals (which means their bioavailability) is controlled by highly specific, strong complexing ligands exuded by the marine phytoplankton. This rather new research line inspired soon the formulation of a new paradigma, according to which both growth rates and species composition of primary marine organisms are affected by trace metal levels now orders of magnitude lower than conventionally believed. One consequence of this statement was the insight that the natural capacity of natural waters to assimilate metallic contaminants may be actually affected at concentrations much lower than was thought reasonable.

When determining the species distribution and composition of metals in a hardwater eutrophic lake, Odzak et al. (2002) found that *DGT-labile* **Cu** and **Ni** species (DGT = '**D**iffusive **G**radients in **T**hin Films'; see more details in section 5.5.3) were mainly organically complexed (as determined by 'Ligand-Exchange' / 'Differential Pulse Cathodic Stripping Voltametry' (DPCSV)) and constitute up to about 15-25 % of the total dissolved metal concentration. Of the total dissolved **Zn** about 30- > 90 % was found in a DGT-labile form (of total Mn about 50-100 %), of which less was probably organically bound/complexed. A larger fraction of the DGT-labile Zn occurred in the hypolimnion of the lake, probably due to strong ligands available in the productive surface water. The authors conclude that the combination of measurements of dissolved and DGT-labile species may better allow us today to elucidate those mechanisms controlling the cycling and bioavailability of metals in a lake.

To assess the aqueous speciation and bioavailability of **Zn** in runoff water, Karlen et al. (2001) used in combination with a water-ligand model ('MINTEQA2') and a humic aquatic model ('WHAM'), a standard growth inhibition test (algae *Raphidocelis subcapitata*), and a Zn-specific biosensor with the bacterial strain *Alcaligenes eutrophus*. Their models and test results showed that a majority of the released Zn occurs as free hydrated, bioavailable Zn^{2+} ion.

We know that many trace metals form also complexes with dissolved *refractory (less degradable) organic substances (ROS)*, why metal mobility in aquatic systems strongly depends on the kinetic and thermodynamic stability of these compounds. High values of complexed metals are usually found for Cu but not for Zn, while Cr^{3+} forms kinetically stable ROS complexes. Empirical studies also show that there is no positive correlation between DOC and the complexed metal fraction in natural (and waste) waters indicating that it is not the DOC of the ROS *per se*, but different functional groups present on these organic substances, which are important for metal complex formation (ref. in Heumann et al., 2002).

In a recent survey, Heumann et al. (2002) observed different complexed fractions for total **copper** and **zinc** indicating differences in the complexing capacity of ROS for different metals. As an example, Cu was almost totally complexed by ROS in wastewater, but only up to 39% in natural brown water samples. Correlating structural data obtained by ^{13}C NMR (Nuclear Magnetic Resonance) measurements for ROS with the corresponding complex formation helped to elucidate the reason for these differences. By evaluating the NMR spectroscopic data, Heumann and coworkers succeeded to relate a decrease of the C-O binding fraction in ROS's to a decrease of Cu complexes, whereas the aliphatic binding character increased with decreasing Cu complexation. It is well known that the thermodynamic and kinetic stability of metal-ROS complexes is an important property in controlling the distribution, mobility and bioavailability of metals in aquatic systems (see Fernando 1995, and references given in Heumann et al. 2002, for methods to determine stability constants of metal-ROS complexes). According to our present state-of-knowledge, if a complex can be judged *kinetically labile or stable* depends on the speed of exchange of a ligand between the dissolved "free" metal ions and those in a complex. Taube (1952) (ref. given in Heumann et al., 2002) defined a complex kinetically stable if the reaction time for exchange of ligands is more than 1 min. Time scales of the ligand exchange rate for kinetically stable metal-ROS complexes are supposed to be hours and days rather than minutes. The authors further point out that transition metals, including Cu, Zn, and **Ni** with a d^3 (high spin) or d^6 (low spin) electron configuration are known to form kinetically stable complexes even under thermodynamically unstable conditions. By means of experiments with isotopically enriched spikes of metal ions (here of Cu^{2+} and $\mathbf{Cr^{3+}}$), it is possible to determine kinetically stable metal-ROS complexes.

The basic theory of the isotope dilution technique in short: an excess amount of a metal ion in its natural isotopic composition is added to a ROS-containing water sample to completely saturate all ligand binding sites. After equilibration, the metal-ROS complexes are separated by size-exclusion chromatography (SEC). An identical isotope distribution in all fractions enables the identification of kinetically labile metal-ROS complexes, because exchange equilibration occurred between the isotopically non-enriched metal ions first bound by ROS, and the isotopically labelled free ions of a spike. In contrast, isotopic compositions similar with or identical with the natural isotopic abundances, indicate kinetically stable complexes. This means that the rate of free metal ions exchange can be used as a measure of complex stability. The isotope-dilution experiments by Heumann et al. (2002) showed that different Cr(III)-ROS fractions can be either kinetically labile or stable, probably due to different structural elements and or functional groups in the corresponding ROS fractions. This was in contrast to results obtained for Cu, where total exchange equilibration with free ^{65}Cu ion was observed for all Cu(II)-ROS complexes of different molecular size, indicating kinetically labile complexes at least within the given test period (of 2 days).

Mobility and transport of trace metal ions in natural waters are strongly regulated by the complexation with humic substances (HS), and by corresponding ionic, hydrophobic and electron donor-acceptor interactions. But so far, only the *thermodynamic stability*, expressed by complex stability constants, is almost exclusively used and supposed to be decisive for the distribution of metals in HS systems. However, and as mentioned before, kinetically *stable metal/HS complexes* can form even under thermodynamically unstable conditions. Marx and Heumann (1999) earlier performed isotope-dilution experiments to investigate the kinetic stability of **Cr(III) complexes** with humic substances (HS). In short, HS solutions of different origin have been spiked with ^{53}Cr(III) and ^{65}Cu(II) after saturation of HS with Cr and Cu of natural isotopic composition and finally the formed complexes separated and assessed by ultrafiltration, **H**igh-**P**erformance-**L**iquid **C**hromatography and **I**nductively **C**oupled **P**lasma-**M**ass **S**pectroscopy (HPLC/ICP-MS), and SEC. Distinct differences observed in the isotopic composition of Cr in the permeate of the ultrafiltration, when compared with the unseparated solution, suggested the occurrence of kinetically stable Cr(III)/HS complexes. The observed kinetic stability can be explained by the d^3 electron configuration of Cr^{3+}. This was in contrast to the Cu isotopic composition, which was identical in the permeate and the unseparated solution, confirming that a total exchange of Cu^{2+} ions took place between the free Cu^{2+} and HS- complexed Cu ions, hence indicating the formation of labile Cu/HS complexes (at least within the experimental time of 1h). The observed differences in the isotope ratios found in the permeate and unseparated sample also indicate a different distribution of molecular sizes and a great variation of functional complexing groups existing in different HS fractions.

The experimental results presented by Marx and Heumann (1999) have been the first definitive evidence for the existence of kinetically stable Cr/HS complexes. The experiments further proved the *long-term stability* of these complexes, which also means that these metal forms show a strong **mobility** in environmental systems after having been formed. SEC results also indicate that high molecular Cr(III)/HS complexes seem kinetically more stable than low molecular complexes.

As most surface waters are oxygenated, the *role of sulphides* for aqueous metal speciation has still received only little attention. Their persistence in oxic waters may be partly due to the formation of relatively stable metal sulphide complexes with large binding constants, but may also indicate their origin from sulphides from sources like sewage works, dams or wetlands. Sulphides have been observed at nano- and picomolar levels even in oceanic waters.

Rozan et al. (1999) recently used a square wave voltametric procedure to identify and semiquantify metal sulphides of **Cu, Zn** and Pb in oxic river waters and their pH-dependence, in combination with ultrafiltration (with a

3000 MW cutoff) to evaluate the size fractionation of these complexes. In natural river water very low free Cu^{2+} levels of 10^{-10}–10^{-14} M have been found probably due to the occurrence of dissolved organic (humic) matter. Voltametric sulphide peak measurements in river waters (pH ≈ 6.8) after a 10 min purge were assumed to be from Co, Fe, Mn and **Ni** (bi)sulphides. Lowering pH down to pH < 5.2, the observed increasing sulphide peak was supposed to originate from dissociating Zn and Pb sulphide complexes and soluble FeS, while down to pH values ≈ 2.8, the observed sulphide peak is thought to be solely composed of S^{2-} mainly derived from the dissociation of CuS. Indeed measuring river water samples revealed no free sulphides indicating that formation of metal sulphide complexes was responsible for the observed sulphide persistence in oxic water. The majority of sulphides occurred as Cu and Zn complexes probably due to their strong binding constants preventing them from oxidation and dissociation in these slightly acidic waters. Although *dissolved metal sulphide complex stabilities* are assumed to follow the order: Fe > Mn > Zn (> S^{2-}) > Cu, Cu was more bound to sulphide than Zn. Overall Cu sulphides accounted for about 30 % of the dissolved metal portion, whereas Zn sulphides remained < 10 %, probably due to the greater amount of Zn present in the river water.

Size fractionation uncovered the influence of the sources of the metal sulphide complexes. The authors speculate about the presence of > 3000 MW size metal sulphide complexes, and explain their occurrence by an organic growth of so-called 'metal sulphide clusters' on the sulphide surface. It is supposed that various organic groups, like phenyl ligands, may bind to these surfaces and hence increase sulphide stability, e. g. against dissociation at lower pH levels. In order to study the oxidation kinetics of these metal sulphides, their losses were studied in closed reaction vessels over a period of 10 days. Regression analysis indicate that first-order kinetics prevail. Table 5.4 shows the rate constants calculated for the identified metal sulphide complexes and corresponding *half-lives* demonstrating greatest resistance for Cu and Zn sulphides. In summary, the experiments by Razon et al. (1999) confirm that metal sulphide complexes *persist* in oxic surface waters, which could be relevant for the speciation of Zn and Cu in small and medium-size rivers with residence times of a few weeks.

Table 5.4. First-order oxidation rate constants and resulting half-lives for metal sulfides in natural river water (from Rozan et al., 1999)

	rate constant (h^{-1})	half-lives (days)
MHS^+	6.4×10^{-3}	6.5
Zn sulphides	2.5×10^{-3}	16.7
Cu sulphides	1.9×10^{-3}	21.9

5.2.2 Adsorption versus bioavailability

Aqueous speciation of trace metals in freshwaters deserves thorough investigation, in particular regarding the question of the *rate-determining step* in uptake processes. In this context, it is assumed for trace metals that the *free* M^{2+} or *total labile M(II) activity* represent the biologically most relevant parameters. According to Jansen et al. (1998) who intensively studied the equilibrium speciation and the labilities of Zn complexes in European river waters by means of DPV/SV (**D**ifferential **P**ulse **V**oltametry / **S**tripping **V**oltametry), complex species which are labile on the effective timescale of biouptake processes are also bioavailable by definition (compare section 5.1.2). However, if such complexes really contribute to the supply of Zn(II) to a cell surface, which selectively takes up the complexed metal, depends on the flux-determining step, e.g. on the *transport through the supplying medium* or *uptake of* Zn^{2+} *at the surface*. In general, complexation between a free (hydrated) metal ion (M) and a ligand (L) can be described as:

$$M + L \quad \underset{K_D}{\overset{K_A}{\leftrightarrows}} \quad ML \tag{5.1}$$

where k_A and k_D are the *association and dissociation rate constant* of the complex ML, respectively. Complexation equilibrium is then defined by the *stability constant*:

$$k_{cond} = C_{ML} / C_M C_L \tag{5.2}$$

where C_{ML}, C_M and C_L are the bulk concentration of the complex, the free metal and free ligand, respectively.

We know that rates of metal complexation reactions in aqueous systems are controlled by the rate of removal of a coordinated water molecule from the *inner hydration shell* of the respective metal ion. The resulting *dehydration rate constant* k_W is directly related to the *association rate constant* k_A of the overall complexation reaction (in eq. 5.1). The corresponding k_W value for Zn(II) has been found to be 10^7 and $10^8 s^{-1}$, and the k_A value in the order of 10^8 M^{-1} s^{-1}. For complexes with known diffusion and stability coefficients (k), it is now possible to make theoretical predictions about their labilities for a given effective time scale (t) and diffusion layer thickness (δ).

It is generally assumed that complexation of metal ions by organic ligands may lower their affinity for surfaces and enhance their mobility, which may for example ease their passage through wastewater treatment plants. In addition, complexation is supposed to *diminish* metal toxicity. Today we can distinguish by means of various electrochemical and competitive ligand exchange techniques between *labile metal species* (e. g. free metal cations, inorganic complexes, and weak metal-organo complexes with conditional stability constants $K_{\text{cond}} < 10^5$), *moderately strong complexes* (e. g. metal complexes with humic material and K_{cond} of 10^5-10^{12}), and *strong complexes* (e. g. by polydentate ligands with $K_{\text{cond}} > 10^{12}$).

Voltametric experiments done by Jansen et al. (1998) suggest the existence of labile Zn(II) complexes with stability constants in the range of $10^{6.4}$ and 10^7 M^{-1} in many river waters. These values are substantially higher than those found for commercial humic acid complexes ($10^{5.0}$ – $10^{5.5}M^{-1}$) at comparable pH and metal-to-ligand ratios. However, theoretical reconstruction of the labilities based on association/dissociation rate constants confirmed the experimental results. In the investigated river water samples it was found that 30 % of Zn was present as free metal ion and 70 % as voltametrically labile Zn(II) complexes. Concerning the bioavailability of Zn in these waters, one has to consider the rate-determining step. In case that transport of Zn(II) in solution may limit its biouptake rate, the *coupled diffusion* of free and complexed Zn will be flux determining. If so, the flux of Zn(II) will be reduced by about 50 %, if 70 % of Zn(II) is complexed, according to Jansen et al. (1998). However, if the actual uptake of Zn at the *water-cell interface* is considered as flux-determining, the flux is directly related to the concentration of the free Zn^{2+} ion (compare also with section 5.4.7.1 and Figure 5.1 below).

5.2.3 Competition between aqueous and solid phases

Geochemical *speciation codes* (like 'MINTEQ') are now frequently used to show which solid phases control the concentration of a metal, e. g. in soil/sediment porewater systems (Yanful et al. 1999). Coupling *aqueous speciation* with *surface complexation models* represents the state-of-the-art in metal adsorption modelling today (see also section 5.4.7 and 5.5.2, below). But still only few sorption studies treat the adsorption of a metal on natural particles as a process of partitioning among a number of different discrete phases.

Radovanovic and Koelmans (1998) developed a conceptual k_d model called 'SWAMP' (**S**ediment **W**ater **A**lgorithm for **M**etal **P**artitioning) as a function of aqueous and solid phase characteristics.* The model uses the concept of *additivity* of discrete site binding of free and complexed metals to solid phases, accounts for *electrostatic effects* and *competitive adsorption* and includes also *aqueous phase complexation* (see section before). Sorption site concentrations between 10^{-6} M (lakes) and 10^{-4} M (rivers) are usually reported in the relevant literature (references given therein). Adsorption sites considered in the model are Fe oxyhydroxides (OH-sites), Mn oxyhydroxides (OH-sites), and organic matter sites (type A COOH-sites and type B OH-sites), as well as free metal ions and hydroxy complexes (MOH^+ and $M(OH)_2$) as adsorbates, and Ca^{2+} and Ca hydroxo complexes as main competing species. k_d values obtained from suspended solids and aqueous phase measurements followed a rather constant trend at each sampling site (lake and river waters): Pb > Cd > **Zn > Cu, Ni**. Comparing this order with the sequence of *metal hydrolysis constants* (Pb > **Cu** > **Zn** > Cd > **Ni**) one would say that Cu seems less reactive towards particles than expected from the hydrolysis constants (opposite to Cd). However, considering the known strong affinity of Cu to dissolved organic ligands (DOM), the observed trend seems reasonable. Best fits for k_d by the 'SWAMP' model were achieved for Cu and Zn. According to the modelling data, sorption of second-order hydrolysis metal species to Fe oxyhydroxide, followed by free metal sorption to Mn oxyhydroxides, in competition with Ca^{2+}, is supposed to be a major significant process of solid phase formation occuring in these waters, consistent with the fact that mono- and divalent cations (Cu, Zn, Ni) preferably associate with Mn oxides (including Mn oxyhydroxides). The relative contribution of the selected solid binding phases for Zn were 69.6 % (Fe), 27.1 % (Mn), and 3.3 % (C_{org}), and for Cu 99.2 %, 0.7 % and 0.1 %, respectively. From the obtained data, the authors conclude that *multiple solid*

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* k_d = distribution coefficient

phases appear to be relevant in modelling metal uptake processes. In contrast, complexes with DOM seem to be most important for Zn and Cu in aqueous phases. It was summarized that surface and solution complexation equilibria can explain the major part of the variation observed for k_d in freshwaters.

Also Lofts and Tipping (2000) used a similar predictive chemical speciation code (WHAM-SCAMP) and found a good agreement between observed and model-predicted $\log k_d$-values for **Zn**. For **Ni** (and Co) the predicted k_d values were higher than the observed ones, whereas the opposite was the case for **Cu** (and Pb). The authors resume that the differing results may depend on (1) the extent of *Ca and Mg competition* for available binding sites, on (2) the chemical nature of the measured particulate metal, (3) on the efficiency of the solid-solution partitioning method, and on (4) the strength of the Cu-organic matter binding. But they conclude that the used model shows a good promise to predict the solid-solution partitioning of metals in aquatic systems.

In an estuarine water, Wen et al. (1999) found a significant correlation between the colloidal metal fraction and the colloidal organic carbon fraction in the water phase suggesting *colloid-bound metals* as a result of metal-organic complex formation. In particular, iron was bound to high-molecular weight colloids, whereas **Cu, Ni** (and Pb) were more associated to colloidal low molecular weight compounds. In addition, *partitioning coefficients* for metals distributed between colloids and true solution (K_c) were higher than between particles (K_p) and true solution (exept for Fe), confirming a strong binding intensity of these metals to macromolecular colloidal organic matter.

Lu and Allen (2001) used batch adsorption experiments with suspended particles and river water to investigate the effect of many factors that influence the partitioning coefficient (k_d) for **Cu**, including pH, total suspended solids (TSS), total copper concentration, dissolved organic matter (DOM), particulate organic matter (POM), hardness and ionic strength. They found *organic matter binding sites* both as dissolved and solid phases as most important in controlling the partitioning of Cu, beside other major factors such as pH and TSS. Measured partitioning coefficients proved to be independent of total Cu concentrations as long as TSS was high, but increased at low TSS, as expected.

Previous studies on the speciaton of dissolved **nickel** (Ni) in surface, waste and runoff waters by *competitive ligand exchange* (e. g. CSV coupled to a chelating resin) indicate complexation of this metal by an extremely strong ligand. Competitive ligand exchange techniques are increasingly used to estimate concentration and stability constants of dissolved or particulate organic carbon metal binding ligands. Significant concentrations of strongly complexed Ni have been observed in many estuarine systems. Most of these estuaries receive industrial wastewaters containing *Ni ethylenediaminetetraacetate* ($NiEDTA^{2-}$), which is supposed to be that strongly complexed Ni species. To further identify this ligand, its source and fate, Bedsworth and Sedlak (1999) performed simultaneous measurements of $NiEDTA^{2-}$ by high-performance liquid chromatography (HPLC) and cathodic stripping voltametry in combination with a chelating resin column in water samples from the San Francisco Bay. Wastewater analysis indicated that $NiEDTA^{2-}$ accounts indeed for the strongly complexed Ni in waste effluents. Equilibrium speciation calculations suggested, what we already know from other study areas, that even other metals, like **Cu**(II), **Zn**(II), and Pb(II), are similarly discharged as *EDTA complexes* from wastewater treatment systems (compare for more details previous AF-MFG technical reports on this particular issue). Also *seasonal variations* have been observed in Ni speciation due to the discharge of stable $NiEDTA^{2-}$complexes from incoming wastewater, and of weaker complexes from surface runoff, leading to higher concentrations of strongly complexed Ni in summer, when surface runoff discharges decrease.

The authors also found that dissolved Ni (i. e. complexed Ni) in the San Francisco Bay decreased with increasing *salinity* from ~ 160 nM down to 5 nM. Also, the *dissociation rate* of $NiEDTA^{2-}$ seemed slow relative to its residence time and sorption unlikely under the prevailing conditions. It seems most likely that part of the *metal-EDTA complex* is converted to *chloro-complexes* when mixed up with seawater. From this, the authors speculate that a substantial part of complexed metals may pass wastewater treatment plants in an unreactive form, which however may dissociate into more bioavailable and potentially toxic metal-chloro forms after released and mixing with seawater. The authors resume that the presence of stable *nonbioavailable metal complexes* should be more considered in geochemical modelling studies, especially when developing site-specific water quality objectives (see also chapter 8).

5.3 In groundwaters

Metal speciation studies in groundwaters are still rather scarce when compared to those in surface waters, mainly due to the lack of macroorganisms, and of non-equilibrium conditions, making it difficult to assess which metals are truly bioavailable. Thus, health criteria developed for metals in groundwaters to be used as drinking water are still based on total concentrations. However, there is an increasing number of studies *concerned with the rehabilitation of metal-contaminated sites (soils, groundwaters), where speciation provides the information needed to find proper technical measures, to reduce metal mobility and bioavailability. This type of research also considers site characteristics and their influence on long-term metal mobility, e.g. to promote the natural attenuation capacity in contaminated groundwater aquifers.*

Information on the speciation of metals in groundwaters is still rather scarce when compared to surface waters. This may be partly because of the fact that bioavailability aspects seem to be of minor importance due to the lack of macroorganisms. However, we know today that groundwater systems (aquifers) are a vital habitat for many *microorganisms*, which can interact in a still rather unknown way with abiotic groundwater parameters and the speciation of metals. We also know that solubility and partitioning reactions of trace metals in groundwaters are more characterized by long-term, non-equilibrium conditions, which make it difficult to describe or predict the particular chemical form, in which metals may occur, when site-characteristics change. For this reason, current health criteria set for metals in groundwater are still based on total concentrations. While one of the main focus of previous groundwater studies was more directed in assessing the influence of acidification on release and fate of trace metals in rather uncontaminated systems, e. g. from natural soils, aquifers or tubing materials, current efforts seem more dedicated to establish the impact of metals released from contaminated sites or landfills into groundwaters (see recent studies published in *Environmental Science & Technology*). There is indeed an overwhelming number of research in the scientific literature now describing the capacity of site-specific properties and conditions in groundwater aquifers, to naturally attenuate the effect of toxic metals, and technologies to strengthen these natural processes by particular on-site engineering measures. As we know that metals occur in different forms, which respond differently to distinct physico-chemical treatments and situations, there is an increasing need to take the true species composition of metals in groundwaters into account, when assessing the natural attenuation potential of soils, or when designing proper technical rehabilitation measures to limit or counteract groundwater contamination (see also section 5.5.7).

As an example, Santos et al. (2001, 2002) recently studied the distribution and speciation of trace metals in groundwater wells along the Guadiamar river (Spain), two years after the “Aznalcollar” mine tailing spill, to follow up the environmental impact of spill-induced metal releases. The use of chemical extraction and anodic stripping voltametry (see section

5.4.4) proved that Cd and **Zn** in the contaminated soil occurred in available forms (i. e. labile and H^+-exchangeable), in contrast to Pb and **Cu**, which existed as less available, strongly inert species. Metals in groundwater wells more far away from the spill were mainly associated with suspended matter and dissolved organic material, while in wells close to the accident an increase of exchangeable metals especially for Zn was observed. The results were consistent with what was expected, i. e. that newly formed metal species are more labile bound than species already present in groundwater systems.

Jensen et al. (1999) investigated the behaviour of trace metals in metal-spiked anaerobic *leachate polluted groundwater* samples at concentrations within ranges typical for landfill leachates. They found a highly varying part of the added "dissolved" metals (Cd, Cu, Pb) bound to *colloidal and organic fractions*, except for **Zn,** which occurred mainly associated with inorganic (carbonate) fractions, and as free metal ion. Also **Ni** was present, like Zn, as a carbonate complex. As most of these metals have been sampled as dissolved fraction (<0.45 μm), the results showed that their behaviour in the environment may actually differ from what is tradionally believed as truly dissolved metal phase.

To investigate the influence of *aquifer material characteristics* on the partitioning (and hence mobility) of **Cu** and **Zn**, Christensen et al. (2000) established k_d-values for these metals by means of batch experiments for sandy aquifer samples at realistic environmental concentrations. The correlation between experimental k_d-values and aquifer material characteristics showed a good agreement with pH at values between 5.3 and 8.9. But the observed mobility of Cu and Zn in the sandy aquifer seemed restricted to pH values < 6, according to the measured k_d-values.

The study of Christensen and Christensen (2000) is an example to demonstrate the limitations we are faced when using *geochemical speciation codes*. The authors tried to determine the occurrence and formation of metal-DOC complexes at different DOC and pH levels in a leachate-polluted groundwater by means of a resin equilibrium method, and to compare results to simulations done by two geochemical speciation models ('WHAM' and 'MINTEQA2'). While the WHAM model gave a good prediction of the Cd- and **Zn**-DOC complexation at pH values between 5 and 8, and likewise for **Ni** at pH 5 and 7, the model overestimated Ni-DOC complexation at pH 8. In contrast, the MINTEQA2 predicts no pH-dependence of the metal-DOC complexation and was for this reason in poor agreement with the experimental results. Instead, the authors estimated the relationship existing between the *conditional complex formation constant* ($\log K_{cond}$) and pH for each particular metal. In using this relationship as an alternative to estimate *metal complexation by DOC*, it was stated that a deviation in the calculated free metal ion activity up to a factor of 2 may occur.

5.4 In aquatic sediments

5.4.1 Introduction

Trace metals discharged into aquatic ecosystems are most likely scavenged by particles and removed to sediments, leading maybe to a situation, where the overlying water is free from elevated concentrations of trace metals, while the sediments have accumulated toxic metals up to levels which may harm biota. Although *sulphides* and *organic matter* have been identified now as one of the main factors for buffering the bioavailability of metals in sediments, toxicity may not be seen even if these substances become exhausted. This will imply the existence of still other binding phases, e. g. of dissolved or colloidal Fe or Mn oxides, which in addition contribute to the reduction of metal bioavailabilities. Metals entering the aquatic environment and associated with surface sediments are subsequently subjected to a multitude of *transformation* reactions occuring during *early sediment diagenesis'*, leading among others to mineralization and the formation of secondary minerals (see section 5.5.6). As we also know that metal affinities to different sediment fractions vary greatly among different metals, locations and seasons, the prediction of bioavailability seems even more complex (ref. 13-15 cited in Fan et al. 2002). In particular sulphides have received much attention in recent years as major binding phases for metals, like Ag, Cd, **Cu**, **Ni**, Pb, and **Zn**, in anoxic sediments (see section 5.4.3).

Since the 1980s, various geochemically designed, analytical approaches have been used to relate the mobility and reactivity of metals to their potential bioavailability in sediments. For example, Fe-normalized metal concentrations in Fe-oxide fractions obtained by *sequential chemical extraction* are recognized as metal forms, which can positively correlate with metal bioaccumulation. Also increasing *(SEM-AVS) values* have been documented to correlate with sediment toxicity (see the following sections). Although known for their low selectivity and reproducibility, single and sequential extraction techniques are still widely used today to get a first estimate of the potential risk of metal-contaminated materials and ecosystems, like soils or aquatic sediments (see sections 5.4.4 and 5.5.3). But as all these approaches still produce rather inconsistent results, they have to be considered more as conservative measures, for example to stress the nontoxic nature of a particular sediment, rather than to indicate a particular toxicity level. Chemical speciation of metals increasingly includes also the use of thermodynamic data and calculations coupled with newly developed *surface complexation models* (see sections 5.4.7 and 5.5.4), which however depend on the accuracy of the dissolved metal measurements and on a proper estimate of available sorption sites. Moreover, the use of these numeric modelling approaches may be misleading, as natural systems often are out of equilibrium, but also because biological systems evolved mechanisms to respond to metal stress, which may in turn cause the respeciation of metals.

As the metal bioavailability we observe in sediments is the result of continuous multiple interactions existing between organisms, contaminants and the sediment, geochemical and biological approaches alone will always address just one aspect of these complex interactions.

5.4.2 Today's knowledge on metal speciation in sediment/water systems

A multitude of speciation methods is now available to determine typical metal species, the relative distribution of metals among various sediment fractions, or the kind and kinetics of transformation between different metal forms. In particular, sequential chemical extractions (like the 'Tessier Method') provide valuable information about the strength of binding between a metal and main sediment fractions, and hence on its mobility and bioavailability. However, these approaches do not measure discrete metal species but, at best, a group of species, or a particular metal-binding sediment fraction defined by the used extraction. For this reason, bioavailability of sediment-associated metals is not directly related to the prevailing pattern of operationally defined metal binding forms, in contrast to water-borne metal species. Other approaches, as a complement to chemical extraction, include the measurement of sediment characteristics, like its capacity to buffer protons and electrons (e. g. sulphide-binding, acid neutralizing capacity, or redox potential), thermodynamic and kinetic solubility calculations, and more recently spectroscopic analysis or the determination of the AVS (acid-volatile sulphides).

5.4.2.1 General overview

As pointed out by Reuther (1999) in his review on trace metal speciation in aquatic sediments, the concept of chemical speciation has been established as a practical tool during the past 50 years, to assess the behaviour of trace metals in both natural and polluted sediment/water systems. A multitude of more or less sophisticated analytical procedures is now available and can be applied in an attempt to determine typical metal species (such as the form of binding between the metal and a solid phase), the relative distribution of the metal among the various, most common, species, and the kinds and kinetics of transformation between different, typical species.

Classical chemical extraction is still today the most applied method to obtain information about the speciation, mobility, bioavailability or potential toxicity of a metal. It originated from leaching procedures used first in soil science to assess the *plant-available portion* of trace nutrients (Jackson, 1958). Similar methods have also been developed by exploration geochemists, including a wide range of *extracting agents* to trace ore mineralization sites in surface soils or sediments. Later, environmental geochemists adapted the methodology to elucidate current and past metal contamination events in fluvial and lake sediments, and to get an estimate of the mobilization potential (Förstner, 1995). With the introduction of sequential extraction procedures (Tessier et al. 1979; Tessier and Turner, 1996) and by improving their analytical selectivity and accuracy, *successive leaching of metals from sediments* made it possible to provide information about the relative strength of major sediment metal-solid associations. From this, it remained clear that there is no single extraction or analytical scheme that is good for trace metal speciation in general. Instead, any approach for speciation of metals in sediments must be chosen in relation to a carefully defined problem.

However, many of the currently used chemical extraction techniques are based on the so-called **"Tessier method"** (Tessier et al., 1979, see Table 5.5) and differ only in practical details, such as sample-weight / extractant-volume ratio, extraction time, washing between individual extraction steps, etc.

Table 5.5. Examples of sequential extraction procedures for trace metals in sediments (from Reuther, 1999).

Fraction	Moore et al., 1988	Tessier et al., 1979
Exchangeable ions	-----	1 M NaOAc, pH 8.2
Carbonate-bound metals		1 M NH_2OH HCl in M HNO_3, pH 2
Easily reducible phase (Fe / Mn oxides)	0.25 M NH_2OH HCl in 25% HOAc	0.1 M NH_2OH HCl 0.01 M HNO_3, pH 2
Moderatelyreduciblephase		0.02 M $(NH_4)_2C_2O_4$ in 0.2 M $H_2C_2O_4$, pH 3
Organic / sulphidic phase	0.1 M $Na_4P_2O_7$ (organic material)	30% H_2O_2, pH 2, HNO_3 reextr. in NH_4OAc, pH 2
	2x $KClO_4$ + HCl (resist. sulphides)	
Residual, mineral phase	HNO_3 / $HClO_4$ / HF	aqua regia (HCl/HNO_3, 3:1)

As discussed by Reuther (1999), there is a plethora of methods available today for the physico-chemical speciation of metals in sediments, each of them adapted to a specific problem. These include a variety of more or less sophisticated approaches, from direct measurements using ion-selective electrodes or expensive equipment, like electron microprobe or scanning electron microscope coupled to an energy-dispersive system, over indirect physico-chemical methods to biological methods involving different kinds of laboratory tests or bioassays (see also sections 5.4.4, 5.4.5 and chapter 7 below).

5.4.2.2 Total concentration approach

Among the indirect physico-chemical methods, perhaps the most widely used is the destruction of the sediment matrix by strong acid digestion, followed by determining the "total metal content" in the final solution by means of spectrophotometric analysis, like AES, AAS, or ICP-

MS. However, these total sediment metal estimates do not really reflect the totality of the metals present and it is supposed that only the harshest digestion method is most reliable for assessing residual or true total metal concentrations in sediments (and soils). Obtained total concentrations are usually *normalised* to sediment dry weight (or in some few cases to the content of sediment organic matter). However, it is usually not well defined how large a fraction of the total amount of a certain metal is really extracted and available for analysis, and how the size of this fraction may differ from one metal to another. Moreover, and most relevant in this context, these methods do not take into account *what kinds of metal species occur* in the sediment and, consequently, no information is obtained about the potential future behaviour of the metal, such as its mobility or its chemical and biological activity (toxicity).

5.4.2.3 Partly theoretical approaches to metal speciation

In order to get a preliminary idea of the behaviour of a metal in a specific sediment, it is possible to use various measures of the *sediment's capacity to buffer protons and electrons*, like its *sulphide-binding capacity, acid neutralizing capacity*, or *redox potential*. The theoretical background as well as the measuring methods are summarised by Reuther (1999). In this context it should be mentioned that the rather new AVS (**A**cid**V**olatile**S**ulphide) concept has attracted particular interest, especially in the US, because of its documented ability to assess the bio-available fraction of certain metals in anoxic sediments. The benefits and drawbacks in using the AVS concept, as well as its theoretical justification, will be further discussed in later sections of this report (see especially section 5.4.3 below; and Brumbaugh et al., 1994).

Thermodynamic calculations of the solubility behaviour of principal solid-metal phases based on pH-Eh diagrams may also contribute to the understanding of how a specific sediment type will "handle" a certain concentration of a metal under well defined conditions. For example, the distribution of metals between various complexing inorganic and organic ligands in sediment pore water can be calculated based on the knowledge of the complex-forming stability constants, the ligand concentrations, the pH and the ionic strength of the pore water. However, the distribution of metals among complexes formed with various ligands involves *equilibrium* as well as *kinetic factors*, which means that calculated data do not always correspond with experimental or field measurements. Chemical equilibrium models used to predict metal solubility of solutions in equilibrium with pure minerals do not account for the kinetics of precipitation-dissolution of solids or for the slow reaction rate of certain trace metal ligand exchanges. Therefore, in many occasions, it is today considered more efficient to make direct determinations of some of the critical parameters, such as the concentration of *free metal ions* in the porewater, e. g. by using ion-selective electrodes, anodic stripping voltametry or by separating the low-molecular solutes by means of dialysis prior to chemical analysis.

5.4.2.4 More empirical approaches to metal speciation (chemical extraction)

Sequential chemical extraction of trace metals from sediments is intended – in each specific step – to release metals associated with a specific sediment phase (for more details see section 5.4.4 below). As reactions between solute metals and particle surfaces are heterogeneous (i.e. adsorption, electron and proton transfers), the extraction efficiency is more kinetically controlled and strongly depends on the availability of specific surface areas and the type of reactive sites (i.e. high- and low-energy sites). In general, results of the chemical extraction depend on (i) the extraction time, (ii) the liquid/solid ratio, and (iii) effects based on the pH and buffer/carbonate status of the sample (Reuther, 1999). Faced with the selection of a chemical extraction procedure, a great variety of options have been described, covering various leaching tests, single-step extraction, using different types of buffer solutions, chelating agents or weak acids, and multi-step sequential extraction.

Single-step extraction is mostly used to estimate the availability and potential uptake of trace elements by plants. However, there is no clear relationship between extractability (by any of the proposed buffers or weak acids) and plant availability of trace metals (Reuther, 1999). These methods may, at best, provide some relative data or indication on the potential mobility or availability of metals when comparing different sediments or soils.

Similar to single-step extractions, *sequential extraction procedures (SEPs)* measure no discrete or stoichiometrically defined metal species, but rather a group of species, or a fraction of the total metal content as defined by the method used (operationally defined). The methods usually identify 3-7 different metal fractions, which are dissolved by the use of progressively stronger reagents, and by minimising overlaps between the reagents. The *main binding forms and mechanisms* – and thus, the metal fractions in a sediment or soil that can be separated by sequential extraction – can be described in the following, very simplified way (after Jenne & Luoma, 1977; Salomons & Förstner, 1980):

1. Physical sorption of cations in surface atomic layers and pores, e.g. by relatively weak 'van der Waals' forces.
2. Chemical sorption of ions or molecules with surface ligands of Fe or Mn oxy-hydrates, carbonates, sulphides and phosphates, or by hydrolytic adsorption.
3. Ion exchange by compensation of charges in the mineral lattice with exchangeable cations (e.g. of negatively charged OH-groups in clays and Fe hydroxides, or carboxyl and phenolic groups in organic substances).
4. Precipitation of dissolved compounds.
5. Complexation with dissolved or solid organic matter.
6. Fixation in inert lattice positions of minerals.

Many of the attempts to determine metal speciation in aquatic sediments have as an ultimate goal to assess the bioavailability of the metal under the prevailing conditions or under conditions that are likely to occur in a near future. However, the troublesome fact is that there is no simple or direct relationship between the distribution of particular metal species in a sediment and its bioavailability. While the bioavailability of waterborne metals may be directly related to the prevailing pattern of species for this metal, the same is not true for sediment-associated metals (Reuther, 1999). However, in spite of this general difficulty, some useful "rules of thumb" may be noted. For example, *"acid-volatile sulphide"* (AVS) is still considered to be a key binding phase for metals in *anaerobic* marine and freshwater sediments, controlling pore water concentrations and the bioavailability of certain metals to benthic organisms.

High concentration of metals in the pore water often correlates with a low AVS content in the sediment (Brumbaugh et al., 1994). It has been suggested that saturation of the sediment's binding capacity is indicated at an SEM/AVS ratio >1, a situation which may result in a flux of trace metals into the pore water. However, the sediment-binding capacity for metals can be greater than indicated by AVS, suggesting the presence of other important binding sites, such as Fe/Mn oxides, organic matter, etc. In fact, there are also other labile sulphides present in many sediments, beside AVS. Three forms of *labile sulphides* with complex reaction equilibria were measured by Brouwer & Murphy (1995) in sediments: molecular hydrogen sulphide, AVS and heat-volatile sulphides. Thus, the exclusive use of the parameters AVS and SEM when characterising the bio-available fraction of trace metals in sediments does not seem to be sufficient for all types of sediment. In addition, it certainly is useful to determine total organic carbon in the sediment as well as the metal concentration in pore water, and – if feasible – to determine also the *acid-producing potential (APP)* and the *acid-*

consuming capacity (ACC) of the particular sediment (Reuther, 1999) (see section 5.4.3 below).

Nonetheless, there is a preponderance of evidence showing that the SEM/AVS model is applicable in dynamic, bioturbated and oxidising field conditions due to the enhanced stability of sulphide complexes of copper, cadmium, zinc, nickel and lead relative to the stability of the iron and manganese mono-sulphide complexes. FeS and MnS therefore act as a buffer for the oxidation of the other metal sulphides. When finally the less soluble metal sulphides are oxidised, freshly formed iron and manganese oxides together with the organic carbon coating on sediment particles will act as new reactive surfaces having a high affinity for free metal ions. As such, the concern of remobilisation under oxidised conditions is minimal. See also detailed comments on section 5.4.6 bioturbation, resuspension and bioirrigation.

A pertinent example was given by Zhuang et al (1994), who investigated the effect of aeration on cadmium bioavailability in sediments in a series of laboratory aeration experiments in batch reactors during periods of approximately one month. During aeration the concentrations of metals associated with AVS and with pyrite decreased. At the same time there were increases in the concentrations of hydrous iron and manganese oxides and these materials became increasingly more important in the binding of cadmium. Following the aeration more than 50 % of the cadmium was associated with the extractable iron and manganese components and approximately 2 % of the cadmium released during the oxidation of AVS entered into the liquid phase.

Although we know that different sediments, with a similar quantity of metals, behave toxicologically differently, due to different types of metal binding and *sediment characteristics*, chemical speciation alone may not be sufficient to assess or predict the biological effect potential of trace metals in sediments (Reuther, 1999). As indicated above, the toxicity of many divalent metals in anaerobic sediments, as an example, is controlled by variable amounts of AVS, but by mainly Fe and Mn oxides in aerobic sediments, in addition to organic substances.

5.4.3 The Acid-Volatile Sulphides (AVS) concept

Sulphide, derived form sulphate-reducing bacteria in the pore-water of anoxic sediments, forms relatively insoluble compounds with various trace metals. These metal sulphides can be liberated from wet sediment by *treatment with 1 N hydrochloric acid (HCl). Sediments containing an excess of this "acid volatile sulphide" (AVS) over "simultaneously extracted metals" (SEM) are characterized by very low pore-water metal concentrations, as well as low metal bioavailability and toxicity.*

The theoretical foundation of this equilibrium-partitioning (EqP) derived AVS/SEM concept is that trace metal sulphides with a lower solubility product than Fe and Mn sulphides form naturally, and so displace iron and manganese, whenever entering the sediment. For this reason, divalent metals appear in pore-waters in reverse order of their affinity to AVS: Cu > Pb > Cd > Zn > Ni. Consequently, by simply comparing molar AVS concentrations to the molar sum of "simultaneously extracted metals" (SEM), sediment quality values can be defined.

This hypothesis was validated by a multitude of laboratory and field experiments, including freshwater and marine sediments and corresponding test organisms. In particular, it was shown that the molar AVS/SEM ratio could predict toxic effects independently of sediment characteristics. Identification of sediments not likely to be toxic, based on AVS/SEM and pore-water concentrations, turned out to be extremely accurate, while prediction of sediment toxicity is more delicate and, at least, demands the use of the organic-carbon-normalised AVS/SEM model. Both the US EPA and the European Commission have now proposed the $\sum$SEM/AVS ratio, or the $\sum$SEM-AVS difference, as a measure for the bioavailability of at least the metals Cd, Pb, Cu, Ni and Zn. And yet, a critical review of the most recent research indicates certain operational drawbacks and a lack of method selectivity, which have not been adequately addressed so far, and call for further research .

The AVS method may rather over- than underestimate the bioavailability of metals, because the used acid digestion also leaches metals from non-sulphidic phases, indicating its rather conservative nature. However, some recent studies also show that certain sulphides are not dissolved and that high portions of metals (like Zn and Pb) may not be present as sulphides in the sediment, despite existing high reactive concentrations of AVS in the pore water.

From laboratory studies we also know that some sulphides dissolve rapidly, while others appear unaffected by sediment oxidation, e.g. upon resuspension. Artifacts may occur by the subsequent reaction of HCl-soluble metals with H_2S released during AVS extraction, and their re-precipitation as metal sulphides, resulting in artificially lower SEM values. That extracted Fe in some cases may be higher than extracted AVS also indicates the simultaneous presence of other Fe fractions, like oxides and carbonates. For NiS and CuS, a correlation has been observed between extraction efficiency and surface area, which may indicate that also dissolution kinetics is *important. Thus, conventional metal sulphide extractions are not as straightforward as many researchers still believe them to be. Though the SEM/AVS concept proves useful to predict the potential bioavailability of certain metals, our ability is still limited to extend these predictions to a whole series of environmentally relevant metals. Nonetheless, its predictive power with regard to the non-toxicity of both anoxic and oxic sediments has been clearly demonstrated.*

New scientific data point to the fact that other routes of exposure, like ingestion of contaminated food, or heterogeneity of the sediment around exposed animals, occurrence of microhabitats, adsorption to surface membranes, variable animal behaviour, in addition to the specific experimental design, or the choice of biological response to assess bioavailability, may additionally explain the deviating results observed in more recent tests, when compared to those from previous AVS studies. We also learn that burrowing organisms ingest sulphide-rich sediment, while bivalves filter and retain particles in the digestive tract, making sulphide chemistry of metals in the pore-water less relevant for explaining the actual metal exposure to these organisms. In addition, we know that surface-burrowing worms irrigate their burrows with oxidized overlying water and, thus, enhance their potential pore-water exposure through gills or surface membranes, beside their ingestion of sediment. It should be kept in mind, however, that any metal that is present in the pore-water has the potential to form non-bioavailable metal complexes with other pore-water ligands,e.g. organic molecules, thereby reducing the metal's potential for toxicity.

More fine-scale sampling techniques, like DGT ("diffusive gradients in thin films") make it possible now to document the release of metals from solid phases in discrete locations within the sediment to an extent that was not possible to measure by conventional sediment or pore-water sampling techniques.

5.4.3.1 Development and application

Can the toxicity of metal-contaminated sediments be predicted?

In the middle of the 1980s, interest was being directed by regulatory agencies to the problem of *predicting the toxicity* of sediment-associated metals. That was particularly the case in the USA, when the Environmental Protection Agency (EPA) recognised the challenge of handling the sediment-specific nature of contaminant bioavailability, when attempting to derive technically defensible *sediment quality criteria (SQC)* with a broad applicability (Ankley et al., 1996). This challenged the start-up of numerous scientific investigations, first, some fundamental, nowadays classical, studies during the 1980s (e.g. Kemp & Swartz, 1986; Tessier & Campbell, 1987), and later on, an impressive series of systematic studies during the 1990s, starting with the basic finding by Di'Toro et al. (1990) that AVS plays a significant role in determining the toxicity of metals in anoxic sediments.

Over the years, there have been various proposals to establish sediment quality criteria (SQCs) or standards for protecting benthic communities from metal toxicity. Many of those attempts have focused on measurements of total concentrations of each trace metal in the sediment, and comparing these with corresponding background values, or in some cases where available to an effects-based endpoint (see, e.g., Sullivan et al., 1985; Persaud et al., 1989). However, an important limitation to these types of approaches is that causality between metal concentration and biological effect is difficult to establish because values are based on total rather than bio-available metal concentrations. Thus, for any given total metal concentration, adverse toxicological effects may or may not occur, depending upon the prevailing physicochemical sediment characteristics (Ankley et al., 1996). In 2000, it was clearly stated at a meeting between sediment scientists that *"empirical methods based on total dry weight metals concentrations in sediments should not be used to predict the toxicity from sediment-associated metals"*.

The question may arise, if equilibrium partitioning is a sound scientific base for predicting sediment toxicity from metals? The phenomenon of differential bioavailability of contaminants across various sediment types was thoroughly examined with regard to non-ionic organic chemicals at a workshop held in 1984 by US EPA (Battelle, 1984). It was observed that the toxicity of the organo-chlorine pesticide Kepone was not predictable based upon total sediment dry weight concentrations, but the toxic effects of the pesticide were strongly correlated with its *interstitial (pore-)water concentrations*. In the case of Kepone, it appeared that pore-water concentrations were controlled by partitioning of the organo-chlorine pesticide to organic carbon in the sediment. This observation was important for the recommendation that equilibrium partitioning (EqP) be pursued as an approach for deriving SQC (Battelle, 1984). The EqP approach for predicting bioavailability of non-ionic organics has received extensive peer review, including two assessments by the EPA Science Advisory Board. As a result of the review, this method was recommended as the best currently available for deriving SQC (see e.g. US EPA, 1992).

Recently, USEPA has evaluated the potential utility of the EqP approach for deriving SQC for metals. Some initial studies by Swartz et al. (1985) and Kemp & Swartz (1986) supported the concept by confirming that toxicity of cadmium to amphipods (benthic crustaceans) in marine sediments could be accurately predicted based upon pore-water concentrations of the metal. However, at the time, the sediment partitioning phases controlling pore-water concentrations of metals were not readily apparent. As mentioned above, considerable research has focused upon elucidating sediment partitioning phases controlling metal bioavailability, e.g. through the use of elaborate sequential extraction procedures (see Tessier et al., 1979; Luoma & Bryan, 1981, and Tessier and Turner, 1995). It was established that *key binding phases* for metals in sediments include iron and manganese oxides and organic carbon. However, an important shortcoming with the early approaches of investigation was that much of the work was done with sediments that had intentionally, or unintentionally, been oxidised through procedures such as sample drying. Thus, the techniques were appropriate only for examining metal bioavailability in oxidised sediments. This resulted in an underestimate of the importance of metal-sulphide binding in the anaerobic horizons characteristic of many if not most natural in-place sediments (Ankley et al., 1996).

Acid volatile sulphide as a factor controlling bioavailability of metals in sediments

Since it is well-known that sulphide is generated in anoxic layers of sediments through the activity of *sulphate-reducing bacteria*, it appeared necessary to take into consideration the *formation of relatively insoluble sulphides* of a number of metals of environmental concern (e.g. cadmium, copper, mercury, nickel, lead, silver and zinc). Thus, Di Toro et al. (1990) investigated the significance of sulphide partitioning in controlling metal bioavailability in marine sediments spiked with cadmium. These researchers used the definition of Cornwell & Morse (1987) to identify the fraction of amorphous sulphide, commonly termed “acid-volatile sulphide” (AVS), available to interact with cadmium in the sediments (see in particular section 5.4.4). “AVS” is defined as the sulphide liberated from wet sediment by treatment with 1N HCl acid. Five to ten grams of the wet sediment are usually treated with 150 ml 1 N cold HCl for 60 minutes.

In their studies of cadmium-spiked sediments, Di Toro et al. (1990) found that when the molar concentration of AVS in the test sediments was greater than that of cadmium (i.e. when the cadmium-to-AVS ratio was less than 1), pore-water concentrations of the metal were small and no toxicity was observed in 10-day tests with crustaceans. These results from experiments with marine sediments were later confirmed in studies with cadmium-spiked freshwater sediments (Carlson et al., 1991).

Based on these initial studies, Di Toro et al. (1992) suggested that metal SQC could be derived by directly comparing *molar AVS concentrations* to the *molar sum of the concentrations of cationic metals extracted* together with the AVS (i. e. SEM = **S**imultaneously **E**xtracted **M**etals; the sum of **SEMs**). This postulate attracted a great deal of interest, and several studies were designed by various groups of researchers to further explore the validity of the ratio SEM / AVS in predicting metal toxicities, including metals such as cadmium, copper, nickel, lead and zinc (Green et al., 1993; Casas & Crecelius, 1994; Pesch et al., 1995; Berry et al., 1996). The overall conclusion was that, as in previous studies, significant toxicity to benthic animals did not occur when AVS concentrations exceeded those of SEM. It was also noted that pore water metal concentrations usually were extremely low when AVS concentrations exceed SEM contents.

A series of sediment titration experiments with a variety of anoxic marine sediments were carried out by Casas & Crecelius (1994) in order to assess the response of the sediments to increasing Cu, Pb and Zn concentrations and to critically examine the metal-spiking concentrations that caused an increase in pore-water concentrations. The experiments showed that the *formation of metal sulphides with solubilities lower than that of the iron mono-sulphide* was favoured. Cu, Pb and Zn were scavenged by AVS and retained in the sediment until the AVS was saturated. The AVS and the binding-capacity values of Pb and Zn were similar, but the binding capacities of Cu were much greater than the AVS values indicating that *also other sites* may be important for binding Cu in sediments. Subsequent bioassay experiments verified that it was possible to predict the toxicity of Pb, Zn and perhaps Cu to the polychaete worm *Capitella capitata* in anoxic sediments. Another useful tool for predicting metal toxicity in these sediments turned out to be the ratio between pore-water concentration and the LC50 value for each metal (Casas & Crecelius, 1994).

The theoretical foundation for EqP-theory based SEM/AVS predictions of metal toxicity is that the sulphides of cadmium, copper, nickel, lead and zinc all have lower sulphide solubility product constants than do the sulphides of iron and manganese, which are formed naturally in sediments as a product of bacterial oxidation of organic matter. As a result, these metals will *displace* iron and manganese whenever they are entering the sediment (Di Toro et al., 1992). Because the *solubility product constants* of these sulphides are small, sediments with an excess of AVS will have *very low pore water metal concentrations*, and *no toxicity* due to these metals should be observed (Berry et al., 1996).

Thus, the question asked by the well-known eco-toxicologist S. Luoma in 1989: *"Can we determine the biological availability of sediment-bound trace elements ?"*, had received at least a provisional, positive answer.

Summarized the results of all these studies were consistent with the following predictions based on EqP theory (after Berry et al., 1996):

- when sediments have a metals/AVS ratio of <1.0, sediments will not be toxic, and little or no metal will be present in the pore-water;
- when sediments have a metals/AVS ratio of >1.0, AVS binding potential will be exceeded, and metals will appear in the pore-water, i.e. available to bind with other sediment phases (e.g. total organic carbon) or with biotic ligands such as benthic organisms, or occur as free metal ion;
- non-toxic sediments having metals/AVS ratios of >1.0 may have low pore-water metal concentrations, less than those known to be toxic in water-only tests, or/and metals are scavenged by additional sediment binding phases or AVS contents are very low.

The appropriate fraction of metals to use for AVS normalisation is the SEM, i.e. the metals which are simultaneously extracted in the cold acid used in the AVS procedure. This fraction is appropriate because some metals form sulphides which are not fully labile in the short time required for the AVS extraction (e.g. nickel and zinc) (see section 5.4.3.3).

Use of IWTU for prediction of toxicity of metals in anoxic and oxic sediments

Toxicity predictions based on sulphide binding would use the sum of the molar concentrations of SEM for the divalent trace metals present (Cd, Cu, Ni, Pb and Zn) for comparison with the molar concentration of AVS in the sediment. If the sum of the SEM is greater than that of AVS, metals may occur in the pore-water in sufficient concentrations to be toxic. If the toxicity of the cationic metals in the pore-water is assumed to be additive (see e.g. Spehar & Fiandt, 1986), it should be possible to predict the toxicity of the sediment in the same way as in the individual metal experiments, using the sum of the *pore-water toxic units* or *"interstitial water toxic units" (IWTUs)*.

Theoretically, divalent metals should appear in the pore-water in the *order* of the solubility of their sulphides. The relative affinity of metals for AVS should therefore be: *copper > lead > cadmium > zinc > nickel*. Thus, nickel should appear first in the pore-water in sediments with SEM/AVS ratios slightly >1.0, followed by zinc, cadmium, lead and copper as the concentration of metals increases relative to that of AVS (or as AVS is exhausted) (Ankley et al., 1996). Exactly this trend was, in fact, observed to occur in metal-spiked laboratory sediments studied by Berry et al. (1996).

Simpson et al. (2000b) found that adding Cd, Cu and Zn to a polluted sulfidic estuarine sediment resulted in the quantitative formation of CdS, ZnS and Cu_2S (not CuS!). When the sedimenary AVS (FeS) was exhausted, added Cu displaced Zn and Cd from their binding phases, as did Cd for Zn, confirming that AVS is the most reactive binding phase in anoxic sediments.

However, in oxidised marine or freshwater sediments, where sulphide partitioning is not fully controlling metal bioavailability, other partitioning phases may be more important. The key binding phases for metals in such sediments include iron and manganese oxides and organic carbon. Any specific sample of natural sediment (e.g., a sediment core) is a complex microcosm with more or less sharp gradients of pH, red-ox potential, total organic carbon (TOC) and, consequently, also of AVS. Therefore, the most important partitioning phases for metals may vary considerably between different sediment layers, and hence, the toxicity of sediment metals.

Due to the above mentioned sediment complexity, and hence the difficulties of predicting sediment metal toxicity based on knowledge of even a great number of sediment characteristics, it has been claimed for some time, that direct determination of the metal concentration in sediment pore-water (interstitial water) would be a more efficient method for predicting sediment toxicity. However, it is also known that the toxicity of the metals in pore-water may be modified by dissolved organic carbon (DOC) and by other ligands as well (see e.g. Winner & Gauss, 1986).

Summarizing studies to test the "AVS hypothesis"

The equilibrium partitioning (EqP) approach used to derive sediment quality guidelines, as endorsed by the U.S. EPA, is based on the following two elements (cf. Boothman et al., 2001):

1. Toxic effects of contaminants in sediments may be predicted by comparison of concentrations of contaminants in interstitial waters of sediments with existing data on effects in water-only exposures (e.g., from U.S. EPA water quality criteria).
2. Factors that influence the partitioning of contaminants between dissolved interstitial water (IW) and solid phases in sediments can be used to normalise sediment concentrations to their bio-available fractions.

The EqP approach incorporates the hypothesis that AVS *limits* the activity and therefore the bioavailability of metals in anoxic sediments. The AVS-metals hypothesis uses the relative concentrations of two *operationally defined* classes of components, AVS and SEM, to predict whether metals in sediments may occur as dissolved species in the pore-water and, thus, are likely to be biologically available. It should, however, be kept in mind that the entire quantity of a trace metal dissolved in IW (interstitial water) may not necessarily be bioavailable, since complexes with soluble ligands may not pass biological membranes.

A decade of research, mainly in the USA, has defined and tested this hypothesis. During this extensive research campaign, several research groups (many of which were associated with the U.S. EPA) investigated various aspects of the hypothesis relating AVS and SEM with the bioavailability of trace metals by applying a wide variety of procedures and tests. The trace metals tested included Cd, Cu, Ni, Pb and Zn, both individually and in mixtures. Some of the experiments were conducted on field samples, while others used metal spiked sediments or samples collected in the field with gradients of contamination by metals and AVS. Results have been obtained from many kinds of marine and fresh-water sediments, with respect to their short-term or long-term (e.g. life-cycle tests) toxic effects in tests with various benthic invertebrates (e.g. amphipods, polychaetes, oligochaetes, insect larvae, snails and daphnids) and fish (rainbow trout) (Kemble et al., 1994; Casas & Crecelius, 1994; Berry et al., 1996; Hansen et al., 1996a; Liber et al., 1996; and Sibley et al., 1996).

In order to summarise the essence of the above mentioned studies and, at the same time, provide a basis for validation of the EqP approach to *deriving sediment quality guidelines*, the main conclusions from each of the six cited papers will be given below:

- Each of the tested sediment samples, collected from a river system contaminated by mining activities, contained a complex mixture of metals such as As, Cd, Cu, Pb and Zn. Whole-sediment tests were conducted in the form of long-term exposures (up to 28 days) of four test species (an amphipod, a midge larva, daphnids and rainbow trout). Divalent metals in sediments with molar *SEM/AVS ratios* ≤ 1.0 were not predicted to be bio-available (Kemble et al., 1994).
- Sediment samples were titrated with Cu, Pb and Zn and subsequently analysed for SEM, IW metals and AVS retention. In most cases, metals were not detected in the pore waters until the AVS was exceeded, suggesting that AVS was an adequate measure for the metal-binding capacity of a sediment (Casas & Crecelius, 1994). Main conclusions drawn were:

(i) AVS is a key factor controlling the behaviour of some metals in sediments.
(ii) AVS is the appropriate parameter to be used for normalising Pb and Zn in anoxic sediments.
(iii) AVS may not be the appropriate parameter to use for normalising Cu in anoxic sediment, as binding capacities of Cu were much greater than the AVS values, implying that also other sediment binding sites are important.
(iv) The *SEM-to-AVS molar ratio* is useful for predicting Pb, Zn and perhaps Cu toxicities to the polychaete worm *Capitella capitata* in anoxic sediments.
(v) The *IW-to-LC50 ratio* is also a useful indicator of metal toxicity to *C. capitata* in anoxic sediments.

- Six separate experiments were conducted in which two or three sediments with varying AVS concentrations were spiked with a series of concentrations of Cd, Cu, Ni, Pb or Zn or a mixture of four of these metals. The amphipod *Ampelisca abdita* was then exposed to the sediments in 10-d toxicity tests.

  The results demonstrated that it is not possible to predict the toxicity of sediments spiked with metals using the total metal concentration on a dry weight basis. The relationship between mortality and total metal concentration in the tests was sediment specific. In contrast, the relationships between mortality and SEM/AVS ratio and between mortality and IWTUs were demonstrated to be *sediment independent* in the studies with the amphipod. Therefore, it was believed that the use of SEM, AVS and pore-water measurements of metals in combination are superior to all other currently available sediment evaluation procedures to causally assess the biological implications of these five metals associated with sediments (Berry et al., 1996).

- In a paper, where results from all experiments (till that date) using field-collected saltwater and freshwater sediments were combined with those from all available laboratory spiked-sediment tests using a wide variety of saltwater and freshwater species, Hansen et al., (1996a) further studied the relationship between total metal concentrations, IW metal concentrations, and SEM/AVS ratios and toxicity to the saltwater amphipod *A. abdita*, and compared these with previously published results. Predictions of sediments not likely to be toxic, based on SEM/AVS ratios and IWTUs for all data from freshwater or saltwater field sediment and spiked sediment tests turned out to be extremely accurate (93.3 – 100%) using either or both parameters. While predictions of sediments likely to be toxic were less accurate (19.4 – 79.1%), this approach was deemed to be very useful in identifying sediments of potential concern (Hansen et al., 1996a).

- In a field-validation experiment with zinc-spiked sediments in a natural pond, bioassays were carried out with the midge *Chironomus tentans* and the amphipod *Hyalella azteca* and field colonisation by benthic invertebrates was followed. Sediment samples were taken five times during a one year period and the abundance of different taxa (chironomidae, oligochaeta, nematoda and bivalvia) was evaluated. No substantial effect on colonisation of zinc-spiked sediments by benthic macro-invertebrates was observed. Only oligochaetes (*Naididae*) were significantly reduced in abundance in the highest zinc treatment for two out of the five sampling dates. The AVS concentrations in the sediments generally displayed low temporal variability, especially in samples spiked with high concentrations of Zn, and there was a positive correlation between AVS levels and SEM Zn concentrations in the sediments, suggesting that natural metal sulphides (e.g. FeS and MnS) had been replaced with the more stable (less susceptible to oxidation) ZnS. Overall, AVS normalisation was useful for predicting non-toxicity (i.e. when molar SEM/AVS ratios were $\leq$1.0), but prediction of toxicity was complicated by additional metal binding phases, such as organic carbon, and by differences in the sensitivity of benthic macro-invertebrates to Zn (Liber et al., 1996).

- An evaluation of the AVS model with respect to its potential to predict metal toxicity during long-term exposure to a spiked sediment was conducted using a life-cycle test with the midge *C. tentans*. The results confirmed those of short-term studies, i.e. when the SEM/AVS ratio was $\leq$1.0, the concentration of Zn in the IW remained low and adverse effects on survival, growth or reproduction were not observed. Conversely, when the molar SEM/AVS ratio was >1.0, elevated levels of Zn appeared in the pore water, leading to reduction in survivorship, growth, emergence and reproduction. Thus, the study supported the use of AVS as an important normalisation phase for predicting toxicity in metal-contaminated sediments (Sibley et al., 1996).

5.4.3.2 Refinement of the "AVS hypothesis"

Temporal and spatial AVS variations:

A further refinement of the hypothesis relating AVS, SEM and bioavailability of trace metals, where special attention was given to the *vertical and temporal variation* of these parameters, was made to account for natural processes in field sediments. To assess the significance of these variations in field sediments, a long-term experiment in which sediments spiked with Cd, Cu, Ni, Pb and Zn were deployed in the sea and recolonisation by benthic organisms was investigated (Boothman et al., 2001). The sediment initially contained about 9 µmoles AVS/g dry sediment, and in order to obtain conditions ranging from excess sulphide to excess metals, equimolar quantities of the five metals were added to sediment in nominal total metals / AVS -ratios of 0.1, 0.8 and 3.0 to 1 (0.1 X, 0.8 X, and 3.0 X).

During the experiment, the AVS concentration in the surface layer (0-3 cm) decreased significantly, except in the 3.0 X sediment, due to the formation of the less soluble metal sulphides from reactive FeS. In the high metal dose experiment, in turn, SEM decreased due to diffusion of excess SEM from the pore water into the overlying water. In the subsurface layer (6-10 cm) no decrease of AVS with time was detected. Also the excess metals ([SEM] – [AVS]) clearly decreased with time in the surface sediment layer. On day 119 of the experiment, sediment cores from the different treatments were collected and sectioned in 0.6 cm intervals to allow profiles of metals and AVS to be determined with finer resolution. While the AV S concentrations usually decreased in the top 2 cm (except in the 3.0 X series), AVS typically increased at all sediment depths below about 2 cm in all treatments. Unlike sulphides, the profiles of SEM showed little change in cores below 2-3 cm. Much like AVS, however, metal concentrations decreased substantially in surface sediments down to almost 3 cm depth. The core profiles finally showed that AVS exceeded SEM throughout the sediment column in all but the 3.0 X treatments. In the highest metal treatment, excess metals ([SEM] – [AVS] > 0) were found particularly in the deeper layers (below 3 cm), where SEM exceeded AVS by 10 µmole/g or more. In sediments less than 2 cm deep, though, excess metals were less than 4 µmole/g in all samples and less than zero in some. Thus, it was expected to find metals in IW in the deeper sediment layers, but that metal concentrations in IW in surface sediments should be low or non-detectable (Boothman et al., 2001).

Analyses of IW demonstrated that Cu was not detected in any of the 22 samples investigated. Cd and Pb were detected in a few samples from the 3.0 X series. However, after 119 days, the only metal detected in the IW in the surface (1-3 cm) sediment was Ni, while Ni, Cd and Zn appeared in the subsurface (7-9 cm) sediment IW. Thus, the magnitude of the metal's concentrations followed the order of solubility of their sulphides, i.e. *Ni > Zn > Cd > Pb > Cu*. Metal concentrations in IW decreased with time for all metals except Cu (because Cu was always below detection limit). Despite this decrease, however, concentrations of all added metals (except Cu) exceeded their chronic water quality criteria (U.S. EPA, 1998) for at least the first 56 days, Cd and Zn for up to 85 days and Ni for the entire experiment. The concentrations of Ni exceeded the chronic criterion by two to three orders of magnitude throughout the experiment.

The assessment of the results of the benthic colonisation of the sediments showed that the colonising communities in the 3.0 X series were very similar to those in the control sediments and also similar to the ambient community. Major phyla were identical, the most abundant being Annelida (primarily polychaetes) and Mollusca. Species composition and – in most cases – organism density were also similar in metal-amended sediments and control sediment. Thus, increasing concentrations of metals added to the experimental sediments, within the limits used in the present experiment, had no detectable effect on the benthic communities (Boothman et al., 2001).

These *field experiment results* apparently *contrasted with* what was found in similar cadmium *spiking experiments* carried out in the laboratory (Hansen et al., 1996). In the latter, higher concentrations of excess Cd were associated with decreased species richness, decreased size, abundance and diversity of polychaetes, absence of bivalve molluscs and greatly reduced density of diatoms. Closer examination of the experimental conditions in the field experiment showed that the apparent discrepancy between the chemical composition of the sediment and the biological response most probably was related to the spatial scale of the chemical measurements and the nature of benthic colonisation. Metal concentrations in IW were measured by integrating the sediment layers between 1 and 3 cm deep and between 7 and 9 cm, respectively, while SEM – AVS was measured at every 0.6 cm on day 119. Since the measure of excess metals in the uppermost sediment layer indicated that no bio-available metals would be present, this result was consistent with the biological data, but the result from the analysis of metals in the IW was not fully representative, because of the low precision in describing the vertical distribution of interstitial metals. This fine-tuned vertical distribution of bio-available metals is important for the interpretation of the biological effects, since the colonisation of a sediment, devoid of infauna, obviously starts at the top, and the relatively small organisms that are the first newcomers, mainly stay in contact with the surface sediment layer. Only at a later stage, larger and/or burrowing organisms, which will exploit deeper layers of the sediment, will become exposed to metals in the IW at greater depth.

It should also be noticed that the discrepancy between the laboratory (Hansen et al., 1996b) and field experiment (Boothman et al., 2001) is probably due to the fact that metals that are released from the uppermost sediment layer are readily washed away by *bottom currents* occurring in the field situation, but obviously not in the laboratory (see section 5.4.6). This may counteract the build-up of toxic concentrations of bio-available metals in the very surface of the sediment, where the first colonisation by benthic organisms takes place.

Because biological response to contaminant exposure appears to be very sensitive to the vertical distribution of contaminants, appropriate techniques should be used to obtain sediment samples from the portion of the sediment column inhabited by the relevant organisms (see section 5.5.3) (Boothman et al., 2001). In the cited study, colonisation of benthic organisms was related more to [SEM] – [AVS] in the upper 1 to 2 cm of sediment than to concentrations of metals in IW at depths of 1-3 cm. The authors considered this to be an artefact of using a sampling device with inadequate vertical resolution; peepers (such as the dialysis-type in situ devices developed by Hesslein (1976)) with better vertical resolution might have revealed gradients of concentrations in the IW.

In general, it seems that meta-stable iron and manganese sulfides can act in the sediment as buffer for the oxidation of metal sulfides (e. g. due to bioturbation), also to form highly reactive surfaces for the adsorption of free metal ions released from the pore water, emphasizing the rather conservative nature of the AVS approach. That the temporal and spatial occurence of AVS and SEM changes in the sediment, due to seasonal variations, diagenetic processes and the acitivity of sediment-living organisms, should not be underestimated, as it can change metal bioavailabilities and so transform formerly non-toxic sediments into toxic, and vice versa.

Organic carbon normalisation:

Another significant improvement of the existing AVS method in predicting not only the lack but also the onset of metal toxicity in spiked and field contaminated sediments has been recently suggested by the group of Di Toro by taking also the partitioning of metals to sediment organic carbon and the effect of AVS into account (Mahony et al., 1996; Di Toro et al., 2002). In this context it is assumed that toxicity occurs if the excess SEM goes beyond the binding capacity of the organic carbon present in the sediment. The authors use marine field sediment data from Long et al. (1998) (reference given), who compared the capability of empirically derived SQG and the SEM/AVS method to predict organism mortality observed in field-collected sediments. When drawing up the available data on a log-normal scale, the authors found that both methods, i. e. the average ERM[2] ratio (ERMR), or the ∑SEM/AVS, are not very useful to predict sediment toxicity. For the average ERM ratio, the uncertain range, where no statistically significant mortality (at a 24% level) was observed, included more than 55% of all data, while for the ∑SEM/AVS ratio the predictive power was even less. Mortality was even observed at SEM/AVS ratios of less than one, which contradicts the AVS-concept. Later, it was shown that the observed toxicity was not due to metals, why the authors conclude that the AVS concept will fail in field sediments where metals are not the only cause of the observed toxicity.

The authors continue to evaluate literature data from toxicity tests using laboratory-spiked marine and freshwater sediments, now including the organic carbon fraction, in addition to the marine field data. When they again compare organism mortality with the average ERM ratio, it became clear that it is not feasible to reliably predict toxicity in these spiked sediment samples by the ERM methods, as the uncertain range included more than 75% of the data. However, the statistical evaluation of the metal-spiked sediment data confirmed that the SEM/AVS ratio could reliably predict the lack of toxicity at SEM/AVS < 1, with an uncertain range covering only 28% of the data. Also when Di Toro et al. (2002) combine the spiked and field-collected samples and compared the average EMR and SEM/AVS ratios to the observed mortality, again the ERM ratio failed to present reliable predictions, in contrast to the SEM/AVS method, which succeeded to reliably predict the absence of metal toxicity in these sediments. But to reliably predict also the ocurrence of toxicity at SEM/AVS > 1, so the authors conclude, the theoretical basis must be further improved.

According to Di Toro et al. (2002), the LC50 of a sediment-bound metal can be expressed by:

$$C_s = K\textit{p}\ \mathrm{LC50} \qquad (5.3)$$

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[2] An ERM (effect range median) is defined as the concentration of a chemical in sediment above which effects are frequently or always observed or predicted amongst most species.)

where C_s is the LC50 sediment concentration (μmol/kg dry wt), Kp (L/kg) the partition coefficient between sediment and pore water, and LC50 (μmol/L) the concentration causing mortality in water only exposures. For metals that form insoluble metal sulfides, equation 5.3 becomes

$$C_s = \mathrm{AVS} + Kp\ \mathrm{LC50} \tag{5.4}$$

The authors further explain that the SEM concentration should be used as the sediment metal concentration C_s, why equation 5.4 becomes

$$\mathrm{SEM} = \mathrm{AVS} + Kp\ \mathrm{LC50} \tag{5.5}$$

From the fact that organic carbon is a well known partitioning factor for metals in water and soils, the authors resume that the partition coefficient in equation 5.5 can be also expressed as

$$\mathrm{Kp} = f_{OC}\, K_{OC} \tag{5.6}$$

where f_{OC} is organic carbon fraction in the sediment, and K_{OC} the organic carbon based partition coefficient.

Using this expression in equation 5.5 yields

$$\mathrm{SEM} = \mathrm{AVS} + f_{OC}\, K_{OC}\ \mathrm{LC50} \tag{5.7}$$

and finally

$$(\mathrm{SEM} - \mathrm{AVS})/f_{OC} = K_{OC}\ \mathrm{LC50} \tag{5.8}$$

To evaluate critical metal concentrations K_{OC} LC50, the authors suggest to use the term (SEM – AVS)/f_{OC} as toxicity predictor, based on measured SEM, AVS, f_{OC} and toxicity data.

When comparing the mortality of the spiked and field-collected sediments to unnormalized excess total SEM (∑SEM – AVS), the uncertainty range still covers about 37% of the data. However, when the organic carbon normalized total excess SEM concentration is used, the range of uncertainty to predict toxicity could be reduced to only 26% of the data, which corresponds rougly to the value predicted by the SEM/AVS ratio.

Surprising for the authors was the fact, that organic carbon normalized excess SEM concentrations can be apparently applied to all the sulfide forming metals (Cd, Pb, Ni, Zn) tested, except for Cu (for which no non-toxic data for positive excess SEM values are available) and Ag (due to the particular role of sulfur-containing ligands for Ag complexation). Concerning the ability to even predict chronic toxicity, the authors further compared data from tests with metal-spiked sediments to organic carbon normalized excess SEM. In fact, the obtained results suggest that the lower boundary, i.e. $\sum$SEM < 150 µmol/gOC, where no acute toxicity was found, is also the boundary below which no chronic effects may occur, in contrast to the uncertainty range of the observed acute toxicity, where chronic effects occured. From this, the authors draw the conclusion that the threshold level for chronic effects may be at $\sum \mathrm{SEM}_{x,OC} = 150$ µmol/gOC.

From the EqP equation $\sum \mathrm{SEM}_{x,OC} = K_{OC}$ LC50, and the corresponding K_{OC} values found for the metals Cd, Cu and Pb, the authors calculate the range for the toxicity threshold (32 – 100,000 µmol/gOC) and compare it to the much narrower range found by using the organic carbon normalized excess SEM concentration (150 – 3400 µmol/gOC). From the narrower range, the authors conclude that there may be an inverse correlation between LC50 and K_{OC}, which would mean that metals with lower LC50s also have a higher K_{OC}. The negative correlation between K_{OC}*s* and LC50s suggests, so the authors, that the toxicity of a metal is inversely related to its affinity for organic carbon, as expressed by its partition coefficient, a relationship, which may bridge the gap to another toxicity predicting model, the Biotic Ligand Model (BLM) (see chapter 6). According to the BLM, the toxicity of a metal (to fish and crustacea) is controlled by the binding strength of metal ions to a biotic ligand (like respiratory membranes), and by the critical biotic ligand concentration required to produce mortality. If the binding strength, so the authors further rationalize, to that biotic ligand is proportional to the binding strength to organic carbon, then metals with a low K_{OC} would tend to have a high LC50, and vice versa.

The applicability of the SEM/AVS model was also recently evaluated by establishing *Receiver Operating Characteristics Curves (ROC)* to compare different approaches/models that estimate the toxicity of metals in sediments (Shine et al (2003). The focus was to test the capability of a method to correctly classify a toxic sample as toxic and a non-toxic sample as non-toxic. ROC curves were constructed by using acute toxicity data from 357 samples chosen from eight sources including freshwater and marine sediments. Species tested were *Hyalella azteca*, *Chironimus riparius*, *Neanthes arenaceodentata*, *Capitella capitata*, *Lumbriculus variegates*, *Helisoma* spp., *Ampelisca abdita* and *Chironomus tentans*. Both the negative and positive predictive capability was examined. From this analysis it became clear that the SEM/AVS model provides an adequate negative predictive power of 97 %, but a low positive predictive potential (of 55 %). As the latter means the likelihood that a sample exceeding the threshold is in fact toxic, toxic effects are not observed in a large number of cases exceeding the SEM/AVS ratio. This is not surprising since both the SEM/AVS threshold of 1, and the SEM-AVS threshold of zero, are not considered to predict toxicity, but intend to provide information about the absence of metal-related toxicity in sediments.

5.4.3.3 Operational drawbacks of SEM/AVS-based approaches

AVS-based *sediment quality values* (SQVs) are currently considered as sediment quality guidelines by the regulatory community. Extraction techniques using cold HCl to measure the concentration of sulphide mineral associated trace metals are widely accepted as a proxy for the potential metal bioavailability in sediments (see references given in Landner 2002, and Reuther 1999). The fundamental principle of the *AVS theory* is built upon the assumption that in sulphide-rich sediments trace metals are predominantly present as sulphide phases and that non-sulphide binding phases like hydroxides, carbonates or organics are insignificant. It is further assumed that the analytical AVS procedure extracts particularly monosulphides and that for every mole of AVS sulphide a mole of metal measured as SEM (**S**imultaneously **E**xtracted **M**etals) is extracted. This is in fact in agreement with many experimental findings that metal ions added to anoxic sediments form discrete metal sulphides.

The AVS-approach can be generally expressed by the following solubility-driven exchange reaction:

$$Me^{2+}\,(aq) + FeS(s) = \text{metal sulphide}(s) + Fe^{2+}\,(aq) \qquad (5.9)$$

Based on this equation, it is assumed that aqueous metal concentrations should not build up and hence sediments are supposed not to be toxic to benthic invertebrates (with regard to metals), as long as there is sufficient FeS(s) available in the sediment to complex all SEM. Only at molar SEM/AVS ratios ≥ 1, no FeS will be available to sequester metals, which now can build up toxic porewater levels. So far the widely accepted theory.

However, **recent research** gave new scientific evidence to question some basic assumptions of the AVS concept (see below). So, since its introduction in the 1990s as a tool for practical risk assessment of contaminated sediments, it has been shown that the SEM/AVS concept still has some operational drawbacks and an obvious lack of selectivity. It became in particular clear, that the HCl-extraction does also dissolve (or not) in a rather unpredictable way some other sulfides in the sediment.

The most common way to measure SEM/AVS is to digest sediment at room temperature for 1 h in either 1 or 6 M HCl. The evolved hydrogen sulphide is trapped in a basic solution by a Soxhlet apparatus, the sediment slurry filtered and the filtrate analyzed for metals. The molar ratio of the acid volatile sulphide (AVS-sulphide) to the simultaneously extracted metals (SEM-metals) is consequently used to predict sediment toxicity (see section 5.4.3.1).

Comparing SEM to AVS, either by ratio (∑SEM/AVS) or by difference (∑SEM-AVS), as a measure of bioavailability was proposed by the US EPA for the metals Cd, Pb, Cu, Ni and Zn. In a recent study, O'Day et al. (2000) re-examined the theoretical support for the SEM/AVS approach, which is based on the following *assumptions*: (1) these 5 metals precipitate from porewater as monosulphides and SEM measures total metal concentrations associated with these sulphide phases, (2) AVS is formed by iron sulphides including mackinawite ($Fe_{1+x}S$), greigite (Fe_3S_4), and amorphous FeS, and maybe minor quantities of ZnS, PbS, NiS and CdS, and (3) pyrite does not dissolve and contribute to the AVS metal pool (see section 5.4.3.3 for more details). Sediments with ∑SEM/AVS > 1 (or ∑SEM – AVS > 0) are interpreted as potentially toxic because HCl-extractable metals exceed the amount of reactive sulphide, assuming that FeS is insufficient in the sediment to react with dissolved metals in the porewater to form less soluble monosulphides, and so precipitate metals from solution. But increasing studies show that the AVS method is not selective, and SEM and AVS are rather operationally defined, including not only easily extractable and sulphidic phases, but also other non-sulphidic metal-binding compounds in the sediment. This chemical non-selectivity adds to the fact that the AVS concept is supposed to be a rather conservative measure of both metal bioavailability and/or toxicity in anoxic sediments.

Cooper and Morse (1998) found that some metals may be present in sulphides poorly soluble in cold HCl, whose solubility is rather subjected to surface area effects. For example, it was particularly shown for NiS that HCl extraction efficiency decreases with decreasing surface area (i. e. increasing grain size). A similar trend was also demonstrated for CuS for the 1 N HCl extraction (see Table 5.6).

Table 5.6. Effect of surface area on extraction efficiency (from Cooper and Morse, 1998)

	serie 1		serie 2		
Metal sulphide	HCl (%)	HNO_3 (%)	HCl (%)	HNO_3(%)	surface area (m^2/g)
CuS* (purchased)	8	92	14	86	0.71
CuS (synthetic)	32	68	12	88	46.31
NiS* (purchased)	23	78	25	75	2.27
NiS (synthetic)	55	45	42	58	8.49

* indicates metal sulfides that were not fully recovered by the combined HCl + HNO_3 extraction

Studies by Cooper and Morse (1998) examined the solubility of *pure trace metal sulphides* indicating that the AVS concept may be most successfully applied to Zn, Cd and Pb contaminated sediments. Laboratory experiments could document that sulphide minerals, like covellite (**Cu**S), chalcocite (**Cu**$_2$S), cinnabar (HgS), millerite (**Ni**S), heazlewoodite (**Ni**$_2$S$_3$), and vaesite (**Ni**S$_2$), are only *poorly soluble in HCl*, in contrast to greenockite (CdS), mackinawite, pyrrothite (FeS), galena (PbS) and spaelerite (**Zn**S), which proved to be *highly HCl-soluble* (see Table 5.7). Although these synthetic minerals do reflect a rather artificial situation, which rarely may occur under real field conditions, the results show that HCl-based extraction methods may in some cases even underestimate the potential bioavailability of certain metals, like Cu, Hg and Ni, in anoxic sediments. We also have to keep in mind that this particular method has even less relevance for oxidised sediments. Here, a high bioavailability will be predicted due to low AVS values, although we know that additional binding phases, like organic carbon and Fe/Mn oxyhydroxides, cause low metal bioavailabilities, thus confirming the rather conservative nature of the method. However, and despite these limitations, the US EPA has recently endorsed the AVS concept as the best available test scheme to date to assess the bioavailability of the above mentioned 5 metals, due to obviously existing "strong theoretical foundations" (O'Day et al. 2000).

In the following, we will give some further examples to further indicate some shortcomings of the AVS concept, which have been recently found.

It even may turn out that the assumptions made so far, may not be justifiable for the 3 metals mentioned before (namely Zn, Cd or Pb). For example, when these metals were added to sediments as metal powder, oxides or solid salt, they did only slowly react with the available porewater sulphide. For this reason, Simpson et al. (2000a) assumed that high portions of Zn and Pb may not be present as sulphide despite high reactive sulphide (AVS) concentrations were measured in the porewater. If *sulphide coatings* would *limit sulfidization of oxidized metal surfaces*, which are soluble during the AVS-extraction, an excess of SEM over AVS would be observed, although such SEM would be actually unavailable. Potential toxicity would be falsly predicted. Caution is therefore needed also here, when extrapolating laboratory results (e. g. from spiking with metals) to studies with naturally contaminated sediments. We also now know that some metal sulphides releasing metals into a more bioavailable phase do not extract in HCl. Simpson et al. (1998) showed that 1 M HCl (30 min) extracts H_2S, amorphous FeS, mackinawite (FeS_{1-x}) and some greigite (Fe_3S_4), but no pyritic sulphides from the sediment.

Table 5.7. Extraction efficiency (from Cooper and Morse, 1998)

	serie 1		serie 2	
Metal sulphide	HCl (%)	HNO_3 (%)	HCl (%)	HNO_3 (%)
CuS*	8	92	24	76
Cu_2S*	25	75	12	88
NiS*	22	78	25	75
Ni_3S_2*	25	75	31	69
NiS_2	1	99	1	99
HgS*	1	99	1	99
FeS (pyrrhotite)	99	1	97	3
$Fe(S)_{1-x}$ (mackinawite)	100	0	99	1
CdS	99	1	100	0
PbS	99	1	100	0
ZnS	100	0	100	0

* indicates metal sulfides that were not fully recovered by the combined HCl + HNO_3 extraction

Simpson et al. (2000a) detected that some model sulphides (CdS, FeS, MnS, ZnS) dissolved rapidly in 1 N HCl (30 min), whereas others (like PbS) dissolved less rapidly (in contrast to the experiments by Cooper and Moore, 1998) and appeared even unaffected by *oxidation* (e. g. upon resuspension).

Again, CuS and NiS dissolved not at all in 1 HCl, and only a minor part (15 and 10 %, respectively) became oxidized after *resuspension*. These metals seem strongly bound to sulphide even under high acidities (i. e. high HCl concentration). The authors could also document a significantly slower oxidation rate for HS^- than for FeS and MnS and consequently suggest an *increasing sulphide oxidation in the presence of metal ions*. It is generally assumed that the rapid oxidation of Fe^{2+} by oxygen produces Fe^{3+}, oxygen radicals and peroxide, the latter characterized by a higher oxidation capacity than oxygen. Further, Fe^{2+} may itself initiate a catalytic process, where "*FeS in the presence of oxygen catalizes the oxidation of itself*". These oxidation processes are much surface- and pH-controlled with elemental S^0 as a major oxidation product (with maximum rates for MnO_2 at pH 5, for $Fe(OH)_3$ at pH 6.5 and for FeOOH at pH 7).

Also experiments recently done by Sundelin and Eriksson (2000) confirmed that even a strong sediment oxidation (during 3-7 months) did not succeed to significantly alter the bioavailability of AVS-bound metals, like Cd, Zn and Cu, in anoxic sediments, despite AVS was reduced, suggesting sulphidic metal-binding phases less soluble in 1 M HCl, in addition to other binding ligands, like organic carbon (see section 7.2.3.1, below).

The determination of SEM by 1 M HCl sediment extraction was also recently questioned by the studies of Mikac et al. (2000) for the insoluble sulphide forming metals Cu, Ni and Hg. Preextraction spikes were simulating the behaviour of metals dissolved upon HCl extraction and recovery was variable, but much lower for **Cu** and Hg than for Cd, **Ni**, Pb and **Zn**. At least for mercury (Hg) it was shown that HCl-soluble metals may react with H_2S released from AVS during extraction and re-precipitate as metal sulphide (e.g. HgS), resulting in seemingly lower acid-soluble metal concentrations and hence SEM. However, Hg *precipitation artifacts* became less significant at low sediment-AVS. Also sulphide precipitation was considerably lower at higher (2-6 M) HCl concentrations. The difference between 1 M and 6 M HCl in extracting Hg from an anoxic sediment was 1-5 % and 50-60 %, respectively, of the total Hg, questioning the use of 1 M HCl at least for extracting Hg in the presence of AVS. As neither the HCl-insoluble nor the HCl-soluble Hg fraction was extractable by 1 M HCl, its use at least for the partial extraction of Hg in sulfidic sediments is highly questionable. More intriguing, there are studies reporting that a general formation of discrete trace metal sulphides does not take place even in anoxic sediments (references given therein). From this, Mikac et al. (2000) conclude that developing extraction methods for known individual metal sulphide phases will be essential to test these informations. Their studies also showed that similar amounts of the metals Fe, Mn, **Zn**, Cd, **Ni**, **Cu** and Pb are extractable by the employed HCl concentration range. Comparable concentrations of Fe and Mn liberated by 1-6 M HCl suggest that always the same sediment phases have been extracted. But as extracted Fe was about 2-3 times higher than extracted AVS, the existence of other Fe fractions, like oxides and carbonates, was indicated.

Morse (1994) (reference given in Mikac et al. 2000) exposed anoxic sediments to oxic seawater and found that the HCl-extractable fraction increased 3-fold within 24 h, most probably due to metals trapped within a sulphide phase not soluble in HCl, but potentially oxidizable into an oxide form, which was then soluble in HCl. Based on their results, Mikac et al. (2000) admit that conventional metal sulphide extractions are not as straightforward as still many researchers believe them to be.

There is increasing evidence by previous studies that HCl-extraction may only assess a more general degree of metal sulphide association, and possibly not metal fractions of Cu, Ni and Hg included into *pyritic phases*. In addition, the relation observed between extraction efficiency and surface area for NiS and CuS indicates that *dissolution kinetics* may play an important role for these metals and have to be considered when designing more appropriate future extraction schemes (see Table 5.6). Further, incomplete dissolution observed for Ni, Cu and Hg sulphide minerals suggests that the total mass of metals may be likewise relevant. From a geochemical point of view, the extremely small concentrations found in natural sediments imply that metal sulphides may exist in a much less crystalline state, possibly not as discrete mineral phases but as coprecipitates with iron, for example.

Although there is still a need to improve the predictive power of the AVS concept also for the bio-availability of metals, like Cu, Ni or Hg, the successful use of the HCl-extraction to predict bio-available Cd, Pb and Zn species points into the right direction.

5.4.3.4 New empirical evidence

When evaluating the potential for sulphide-associated trace metals in estuarine sediments to enter into more bioavailable phases due to major oxidation events, Cooper and Morse (1998) assumed that there is a direct link between the speciation of Fe among sulphide and oxide minerals and the seasonal variability of the flux of total dissolved **Cu** from the sediment. Released trace metals may become complexed by strong ligands (e. g. DOM), or coprecipitate with Fe sulphides, and/or adsorb to the surface of pyrite or AVS minerals, e. g. when aqueous sulphide reacts with aqueous iron or metastable Fe oxihydroxides (e. g. goethite or lepidocrite) to form solid FeS. Continued dissolution of Fe oxyhydroxide and the low solubility of FeS (mackinawite) may lead to high concentrations of Fe and very low aqueous sulphide in porewaters. The lack of aqueous sulphide and consequently the higher residence time (i. e. availability) of oxygen may in turn make the solid sulphide more susceptible again to oxidation. But without a sufficient supply of oxidants, SO_4-reduction may ultimately exhaust the supply of meta-stable Fe oxyhydroxides, increase the HCl-soluble Fe associated with AVS-minerals and possibly lead to an increase of the aqueous sulphide concentration. This may inhibit metal-ligand formation, favour the formation of metal sulphides and allow a rapid *pyrite formation* and concomitant sequestering of trace metals within pyrite by FeS reacting with aqueous polysulphide or H_2S. The authors conclude that by reducing the residence time of oxygen in the sediment, high concentrations of aqueous and solid sulphide can buffer any potential oxidative dissolution of FeS(s) and so help to preserve any trace metals associated with AVS minerals and pyrite in sediments.

However, the use of AVS-Fe as a proxy for sediment instability with respect to sulphide associated metals is based on (i) the fact that the AVS extraction does not separate Fe oxide minerals according to their chemical reactivity observed during early diagenesis, and on (ii) the assumption that metals adsorbed to the surface of AVS minerals possess different reaction kinetics than metals coprecipitated within the AVS matrix. Differences in the *sulfidization* rate of iron oxide minerals will make the degree of sulfidization dependent on the sulfate reduction rate and so again on the concentration of Fe oxide minerals. To give an example, Sonke et al (2002) took organic sediment samples from a nearby peat bog pool, in an attempt to describe the atmospheric history of a former Zn smelter. By means of ‘microprobe analysis’ they could show that metals brought by atmospheric deposition (probably as oxides) were immobilized by an extensive in-situ FeS_2 and **ZnS** precipitation, suggesting indeed a rapid diagenetic transformation of metal oxides into sulphides.

According to Cooper and Morse (1998), the percentage of highly reactive HCl-soluble Fe obtained from AVS analysis can be used as a measure of the sulfidization potential of oxide minerals, and reflects the potential of the occurrence of measureable porewater sulphide. From their results, the authors conclude that a large ratio of “sulfidized” to “oxidized” Fe may indicate that the supply of sulphide exceeds the supply of oxidants (like molecular O_2, Fe^{3+}, or metastable solid phase Fe^{3+}). In summary they emphasize that the potential of sulphides to trap aqueous metals and to inhibit metal-ligand formation, as well as the potential of these sulphides to be destroyed later upon oxidative dissolution, are key factors for predicting the reactivity of trace metals in aquatic sediments. Accordingly, the *ratio of reactive Fe to AVS* may represent a more inexpensive and useful indicator for the potential reactivity of sulphide minerals and associated trace metals in marine sediments (Cooper and Morse 1998).

Lee et al. (2000a) pointed out the complex, dynamic three-dimensional oxidation gradients, which characterize surface sediments, and reminded of the fact that benthic biota interact with these gradients in a variety of ways to obtain food or oxygen. We know that epifaunal and infaunal animals in anoxic sediments maintain an oxic microenvironment in their burrows and feeding zones, e. g. for O_2-respiration and to avoid sulphide toxicity (see section 5.4.6). By means of 18-d laboratory bioaccumulation tests with 5 test species (2 clams and 3 marine polychaetes) selected on the basis of their broad diversity in feeding behaviour and zone, burrowing mode and direct sediment contact, Lee et al. (2000a) tried to simulate and evaluate the effect of realistic metal, AVS and geochemical gradients on the bioavailability of selected trace metals.

Metal bioaccumulation in bivalves occurred at negative (SEM-AVS) values and low porewater metal concentrations were observed at the highest AVS level, although both clams still accumulated significantly more **Zn** and **Ni** than in the control system (also Cd bioaccumulation occurred at (SEM-AVS) < 0 in most treatments). The experimental geochemical results were consistent with the equilibrium partitioning-based AVS normalization approach, which predicts low porewater metal concentrations when SEM<AVS. AVS seems to control porewater concentrations of Cd, Zn and Ni. However, it was surprising to find that the organisms significantly bioaccumulated metals from the sediment, although SEM was only a small fraction of the AVS. Moreover, toxicity seemed to be eliminated, as was shown in previous studies when SEM<AVS. Also surprisingly, the bioavailability of sediment metals increased linearly with total metal concentrations irrespective of AVS or porewater concentrations.

Similar studies (cited therein), which demonstrate bioaccumulation by benthic biota despite SEM<AVS, suggest either *other routes of exposure*, e. g. by ingestion of contaminated food, or heterogeneity of the geochemistry around the exposed animals, microhabitats, adsorption to surface membranes, different animal behaviour, in addition to the particular experimental design, or the choice of the biological response to assess bioavailability, as factors explaining differences between the presented and previous AVS studies. According to Lee et al. (2000a), their results can be best explained if *dietary uptake* from ingested sediments is assumed as the dominant route of *bioaccumulation* by all tested benthic invertebrates. This would also explain why bioaccumulation was better related to extractable sediment-associated metals than to porewater concentrations. Also other studies (ref. given therein) confirm the assimilation of sulphide-bound trace metals by deposit-feeding polychaetes and bivalves (ref. given). In particular

microcosm studies and biokinetic models often report a dietary uptake as the responsible metal uptake route by benthic biota, which may occur over a wide range of (SEM-AVS) values. An example is given to illustrate, how different experimental designs may influence uptake routes. Accordingly, animals exposed to high metal porewater concentrations may not burrow into the sediment, or avoid ingestion of extremely contaminated sediment, or may die before they ingest. Also varying attributes of the particular test animal may affect metal bioaccumulation. To give an example, *burrowing organisms* ingest sulphide-rich sediment, while bivalves filter and retain particles in their digestive tract, so that the sulphide chemistry of the porewater metals has the least impact on actual metal exposure. But also a greater filtration or feeding rate could enhance overall bioaccumulation. We know that the surface burrowing worm *Neanthes* (or *Nereis*) *irrigates burrows* with oxidized overlying water and so enhances potential porewater exposure through gills or surface membranes, beside ingestion of sediment. From the results available so far, one may draw the conclusion that developing sediment quality criteria also requires a much more comprehenensive and profound understanding of the significance of less extremely contaminated sediments.

Lee et al. (2000b) refer in another similar study to the two chemically and biologically most active interfaces in aquatic systems, namely the sediment/water and the anoxic/oxic transition zone, and to the multiple physical, chemical and biological processes that affect porewater metal and AVS concentrations at these interfaces. However, still only few of these processes are considered in current bioavailability concepts. We know for example that *AVS variations with sediment depth* affect both porewater metal concentrations, and the flux of metals from the sediment into the overlying water. More complicating for the present discussion is the fact that most of the previous studies used manipulated artificial (spiked) sediments with mainly only one distinct AVS level (and with discrete AVS phases).

During a 18-day sediment incubation experiment with an oxidized water column, the original AVS concentration decreased by 65-95 % at the surface of the sediment, but remained constant on its initial level with sediment depth. In sediment test series with variable AVS and SEM values, porewater metals increased with decreasing AVS, and with increasing SEM. Porewater Cd concentrations were inversely related to AVS, but not significantly to extractable (SEM) Cd contents, in contrast to porewater **Zn** and **Ni** concentrations. Zn and Ni porewater concentrations were under or near detection limit when [$\sum$SEM-AVS]< 0, but high concentrations occurred when [$\sum$SEM-AVS] was > 0.

Normally, AVS-studies compare sediments of different origins, which may additionally explain why data interpretation is complicated due to apparently different sediment characteristics affecting metals partitioning and bioavailability in a particular way. Also, spiked sediments with unnaturally high metal loads were often used to get the required SEM/AVS variations. Lee et al. (2000b) demonstrated natural AVS minima at the sediment surface, and that (SEM–AVS) values as well as porewater metals changed with depth, in relation to prevailing redox conditions. *Fine-scale sampling* disclosed a distinct vertical AVS gradient causing most pronounced vertical (SEM-AVS) and porewater metal changes at depths between 0-0.5 cm. Although the geochemical characteristics of *"surface sediments"* have long been recognized as an important component for benthic biota, depending on how deep this "surface" is defined and sampled, there is still a need for a standardized way of defining a "surface sediment". Because if the more anoxic sediment is included in that definition, the more likely it will underestimate the (SEM-AVS) at the often *oxidized water/sediment interface*, which is, however, the most relevant habitat for most infaunal organisms. Without providing further explanation of the possible cause of the observed diffusion, the authors conclude that the obtained detailed porewater profiles are controlled by a combination of (∑SEM-AVS) and diffusion of metals into the overlying water, as indicated by the large concentration gradients existing between porewater and overlying water.

By means of DGT depth profiles (see section 5.3), Zhang et al. (2002) could recently confirm the capability of fine-scale sampling to document that metals may be released within the sediment from solid phases in *discrete locations* that are not measured by conventional porewater sampling techniques due to their horizontal averaging.

Lee et al. (2000b) finally refer also to empirical studies, which show that porewater partitioning coefficients $k_{\mathrm{d(PW)}}$ are negatively correlated with (∑SEM-AVS), with most $k_{\mathrm{d(PW)}}$ values ranging between 10^3-10^5 at (∑SEM-AVS)<0 for Cd and **Zn**, and between 10^1-10^3 when (∑SEM-AVS)>0, emphasizing the strong affinity of Cd, **Ni** and Zn to sulphides in relation to other ligands. But $k_{\mathrm{d(PW)}}$ values varied by 2-3 orders of magnitude for a given (∑SEM-AVS) value, probably due to variations in experimental conditions (like equilibration times, metal concentrations or sediment characteristics). Also $k_{d\mathrm{(PW)}}$ values obtained from field sediments were higher than those from the laboratory, due to different equilibration times *(aging!)*. How complex the interactions between metals and sulphides may be, has been demonstrated by the fact that AVS values increased at higher spiked metal concentrations both in surface and deeper sediments, suggesting that spiked metals may be able to retard AVS oxidation.

5.4.4 Chemical extraction and mobility

Chemical extraction is still the method of choice when assessing the environmental relevance of sediment-associated metals. It can give a first estimate of their mobility, provide us with information on changes of reactive sediment compounds (like Mn and Fe oxides, sulphides or organic matter), and elucidate the binding strength of metal-sediment associations. Although chemical extractions are still hampered by significant operational drawbacks, like re-adsorption and re-distribution phenomena or lack of selectivity, recent research confirms their successful applicability when characterizing or classifying metal-contaminated sites.

Chemical extraction is still the method of choice when assessing the environmental relevance of trace metals in sediments (or soils; see section 5.5 below). It rather easily allows to differentiate between *potentially mobile metals* and metals that strongly bind to the mineral lattice, or immobile. In particular, *sequential extraction procedures (SEPs)* are widely used, as they provide valuable informations about the species composition, bioavailability, pH and redox sensitivity of sediment-bound metals, that can not be obtained by total concentrations alone. They also allow us to predict possible changes in bioavailability if environmental conditions change.

However, and this is well accepted today, sequential extraction procedures (SEPs) are fraught with a high degree of uncertainty. At first, the obtained metal-binding phases are *only operationally defined* by the particular selected extraction procedure and may at best represent chemical species or not (cf section 5.4.2.4). Taking this into account, Dodd et al. (2000) studied contaminated anaerobic mud samples by means of 2 SEPs (Kersten and Förstner 1986, and Quevauviller 1998, cited in Dodd et al. 2000), and a Cryogenic **S**canning **E**lectron **M**icroscopy (SEM) method coupled with **E**nergy-**D**ispersive **X**-ray **A**nalysis (EDXA), to check the extraction efficiency, reagent selectivity and possible reprecipitation. By analysing the unleached mud, the most abundant authigenic minerals were in decreasing order: Fe^{2+}-phosphate (vivianite $Fe_3(PO_4)_2{\cdot}8H_2O$) > mixed Fe-**Cu-Zn** sulphides > pyrite > calcite. Also, calcite did not completely dissolve during carbonate extraction, while vivianite began to dissolve in the carbonate extraction step of both SEPs, and was completely dissolved after the respective oxide extraction step. It was further shown that the ammonium oxalate residue still contained abundant Fe oxalate crystals, suggesting that

Fe from the dissolution of vivianite has been reprecipiated. The Fe oxalate dissolved in the subsequent sulphide extraction step. However, no sulphide or metal rich organic matter was found in the residue of the sulphide extraction step.

In this context, Dollar et al. (2001) used a Tessier-type sequential extraction scheme for metals in wetland sediments to study the metal *remobilization potential*, in particular with regard to possible implications of different restoration options. Their results gave a first indication, which metals may be affected by possible wetland reflooding. As **Cr** and **Cu** were mainly associated with oxidizable sediment fractions, these metals do not seem to be mobilised as a result of flooding, but may be even more stabilized. With flooding and corresponding wetter conditions the authors speculate that a concomitant decrease in redoxpotential may lead to the dissolution of oxides and hydroxides and the release of associated metals into the water. As Cd, Pb and **Zn** were mainly associated with Fe/Mn oxides, these metals may, consequently, be subject to remobilisation, if these sediments become flooded. Although only insignificant amounts present in the uppermost sediment, considerable amounts of Zn (beside Cd and Pb) may become remobilized at the same time from exchangeable fractions. By means of a simple mass balance calculation based on the performed chemical extraction, the authors assume that restoring only half of the most extensive part of the wetlands, the ‘Great Marsh’, with about 750 ha, would result in the total loss of the exchangeable Zn fraction (1125 µg/cm2) in 1 year as a result of continuous flooding. From this it was estimated that about 84 t of Zn will be flushed away from the system, causing concentrations in the drainage water flowing into Lake Michigan as high as 5 ppm.

In the following some additional recent examples will be given to shortly underline the still existing *difficulties and errors* associated with the use of chemical extractions, which hamper a scientifically sound interpretation of the obtained data.

Ngiam and Lim (2001) used 3 different multi-step sequential extraction schemes to elucidate the speciation of metals in anoxic sediments. They found that sediment samples were already oxidized during the reducible fraction extracting step, resulting in an *overrepresentation* of metals associated with reducible phases and an *underrepresentation* of organic/sulfidic-bound phases, although more than 70 % of **Zn** (Cd and Pb) were associated with AVS.

When Lead et al 1998 studied the partitioning of metals into suspended particulates from a river, they observed a decrease of the metal binding capacity of the sample material when successively extracted by the Tessier method. However, transmission electron microscopy showed that the biological part of the suspended matter had undergone *significant changes* *during extraction*. This may have affected the original metal binding, and so complicated interpretation of the results with regard to simple mineral and organic phase reactions.

Gomez Ariza et al (2000) compared the feasibility of 3 well known sequential extraction schemes (i. e. the Tessier, Meguellati and BCR method) in sediments and provide an interesting discussion about their benefits and limitations. A more comprehensive discussion about the limitations associated with the use of chemical extraction schemes for metal speciation in aquatic sediments is given in Reuther (1999).

Also the fact that still a multitude of various digestion solutions of varying strength is used to assess the degree of total metal contamination, is contributing to handle current speciation data based on chemical extractants with great care. Sutherland et al. (2001) recently tried to test the capability of different extractants in identifying significant contaminant levels in road sediments. In their study, they included a total four-acid digestion scheme, a microwave-assisted digestion with conc. HNO_3 (USEPA 3051), 0.5 M cold HCl, and 0.05M EDTA (pH 7). The authors summarized that the weak extractants they used (i. e. HCl and EDTA) were most successful to indicate the degree of anthropogenic metal contamination, why they recommend to use these agents more widely.

In a very recent comparison, Hseu et al. (2002) tested the applicability of *different digestion methods* to show, which method was best to assess total metal concentrations in soils and sediments. A great number of samples was subjected to aqua regia and different combinations of concentrated acids ($HClO_4$, HNO_3, H_2SO_4, HF). The results indicate that the 'Baker and Amacher' method (1982) (ref. given therein), consisting of a mixture of HF-HNO_3-$HClO_4$-H_2SO_4, performed best for Cd, Cr, Cu, Ni and Zn, and the 'Reisenauer' method (1982) for chromium, the latter using the same acid mixture but a modified heating process. The classical aqua regia gave also comparatively good results for Cu, Ni and Zn, while total Pb was extracted most efficiently by the so-called 'Burau' method (1982) (also cited therein), where the H_2SO_4 of the above acid mixture is replaced by HCl.

That chemical extraction, despite all these uncertainties, still provides a practical tool for getting valuable informations on the complex dynamics existing in sediments in regarding to the mobility, binding and bioavailability of metals, can be further underscored by the following examples.

According to van den Berg et al. (2001), assessing the mobility of contaminants is an important issue in the environmental risk assessment of soils and sediments. In particular, changes in the level of reactive phases (like Mn and Fe oxides, sulphides or organic matter), as derived by chemical extractions, may give us the necessary information on processes, which influence the behaviour of metals in sediments. The authors could demonstrate by means of a chemical extraction scheme that dissolved metal concentrations in water will not be significantly influenced by dredging operations, due to their obviously strong metal binding strength to solid phases, and/or their fast redistribution over sorptive phases in response to oxidation e. g. of metal sulphides.

There is a vast amount of literature now available, which can demonstrate the successful use of sequential extraction procedures to evaluate the efficiency of different sediment treatment techniques (see in particular recent publications in Environmental Science and Technology). When used before, during and after a particular treatment, changes in metal *extractability/leachability* or possible species transformations are indicated. But it becomes also obvious, from which *sediment pools* (e. g. carbonates, Fe and Mn oxides, organic matter and sulphides) metals are released (see for more details also section 5.5.4) (Chen et al. 2000; Chartier et al 2001).

Clark et al. (2000) argue that size *normalization* during sequential chemical extraction may be a rather safe and easy way to directly compare sediments from dissimilar environments, and may in particular help to answer the question, whether a sediment is a source or sink for trace metals.

In order to integrate Microtox toxicity data (EC50 values), sediment contaminant concentrations and speciation data from sequential extraction, Mowat and Bundy (2002) developed a mathematical algorithm. They calculated a *'toxicity index' (TI)* as an indicator of adverse ecological effects, which can be used to rank metal contaminants according to their particular toxicity. Summarizing, the authors conclude that concentrations using bioavailability data from sequential extraction were found to be the best theoretical predictors of the observed experimental mixture toxicity value.

How helpful chemical extraction can be used, when assessing the toxicity of surface sediments, may be reflected by the following short example. Borgmann and Norwood (2002) observed that sediment profiles of the total metal concentration from a lake near Sudbury, Ontario (Canada), differed from corresponding bioavailability profiles, as derived from chemical extraction. Likewise, **Cu** bioavailability profiles differed from profiles obtained for Cd, Co and **Ni**. In fact, deepest *preindustrial sediment layers* proved to be non-toxic, while the observed sediment toxicity at the surface could be attributed to the dissolution of Ni from labile phases into the overlying water. Chemical extraction so could help to trace the occurrence of different metal bioavailabilities in surface and deeper sediment layers, a fact, which has to be considered when interpretating and comparing toxicity data for sediments taken by different sampling techniques (e. g. by grab or core samplers).

5.4.5 New spectroscopic approaches

Spectroscopic approaches are increasingly used today as direct means to assess environmentally relevant metal species in particulate matters. In particular, X-ray absorption spectroscopic techniques (XAS) have almost revolutionized our present understanding on metal speciation, as they identify and measure discrete metal or ligand species of solid surfaces (of minerals or cells), as well as surface characteristics, which may be critical. By providing the necessary structural information, these techniques present a robust molecular basis for method interpretation and verification, and allow improved understanding of results obtained from other speciation methods (like AVS or chemical extraction). By some of the new techniques, it is possible to distinguish the dominant metal-ligand species in sediment samples, elucidate if metals are really present in the form (e. g. as sulphide or oxide, or as a mixture of both) indicated, e. g. by chemical extraction, and hence establish the true species composition.

New techniques such as ***T****ransmission* ***E****lectron* ***M****icroscopy (TEM) coupled with* ***E****nergy* ***D****ispersive* ***S****pectroscopy (EDS) have been successfully applied in speciation studies to provide information on particle size, morphology, crystallinity and composition and have revealed that sediment particles are associated with biological cells and even surrounded by extracellular structures. It is supposed that these particles act as important metal carriers in the aquatic environment, and as catalyst sites for further particle formation. Obtained results emphasize also the need to work at the micro-scale to identify relevant contaminant-bearing phases in aquatic ecosystems, and to help to understand the role of biological surfaces, as important sites for metal speciation.*

On the one hand, there is a need for rapid and routine sediment contamination and toxicity assessments, which underscores recent efforts by various controlling authorities to establish more standardized testing methods and quality criteria for metals in sediments. However, these test methods and criteria have to be put on a scientifically sound basis. For this reason, there is also a growing demand on the other hand, within the regulatory community, to develop more accurate sediment quality protocols for trace metals. Both can be achieved, for example, by coupling conventional chemical speciation methods more closely to standardized toxicity tests, and more recently, to spectroscopic analysis. This type of more synergistic biological-geochemical approach may successively improve already existing attempts to assess toxicity as a function of metal concentration, sediment type, porewater geochemistry, and may reflect more accurately the true range of geochemical processes, for example responsible for removal or uptake of metals from the porewater. As a matter of fact, new spectroscopic techniques are increasingly considered as a direct means to assess relevant metal species, as they represent a robust molecular basis for method interpretation, verification and for the transferability of results obtained from more conventional chemical and biological tests, among a range of aquatic environments.

In particular, *absorption spectroscopic approaches* have almost revolutionized our present understanding of metal speciation phenomena, as they allow us to *directly identify and measure different metal or ligand species* in solid materials (like minerals and cells) as well as surface charcteristics, which may be critical. This new class of speciation techniques also helps to validate the role of new concepts, like the SEM/AVS ratio in soils and aquatic sediments, or of the more commonly used chemical extraction procedures (see section before). In the following, some typical examples will be presented to illustrate how spectroscopic methods are successfully applied now to elucidate the complex nature of solid metal speciation.

O’Day et al. (2000) tried to combine the SEM/AVS method with invertebrate toxicity testing and X-ray absorption spectroscopy (XAS), to evaluate metal speciation and the ecological hazard of estuarine sediments from the Seaplane Lagoon, San Francisco Bay. This site that has been contaminated by military and industrial activities, but should now be returned to the public and private sector. Bay sediments are characterized by a moderate to low toxicity and by increasing sediment and porewater metal concentrations with depth. SEM/AVS ratios and results from toxicity tests were compared with the molecular binding of metals in sediment solid phases measured by ‘**X**-ray **A**bsorption **N**ear-**E**dge **S**tructure’ (XANES) and ‘**E**xtended **X**-ray **A**bsorption **F**ine **S**tructure’ (EXAFS) analysis, to show whether metals are present as sulphides or oxides, or as a mixture of both. The results confirm that some assumptions behind the SEM/AVS approach may not be fully valid:

1. Of all the metals studied in the sediment, only Cd was exclusively present as sulphide (namely as amourphous or poorly crystalline CdS).

2. About 80% of **Zn** and 50-70% of Mn were present as sulphide phase (most probably as sphalerite ZnS and alabandite MnS), and the rest remained associated with oxides.
3. All of **Cr** and Pb in the sediment was associated with oxide phases.
4. Fe occurred primarily as Fe(II) in pyrite and clay minerals; and
5. none of the metals investigated was coordinated by organic ligands.

Table 5.8. Local structure of Zn in reference compounds, determined by multishell fit of Zn K-edge EXAFS analysis (from Scheinost et al., 2002)

		first shell			second and third shell		
Reference mineral	formula	CN^a and element	R[Å]^b	ό^2[Å^2]^c	CN and element	R[Å]	ό^2 [Å^2]
willemite	Zn_2SiO_4	4.8 O	1.95	0.005	2.9 Zn	3.25	0.009
hemimorphite		4.1 O	1.94	0.006	21.4 Zn	3.33	0.028
$Zn_4Si_2O_7(OH)_2H_2O$	$ZnAl_2O_4$	4.4 O	1.97	0.005	14.5 Al	3.41	0.005
gahnite							
Franklinite		4.0 O	1.96	0.003	12.0 Fe	3.51	0.007
$(Zn,Fe,Mn)^{II}(Fe,Mn)^{III}{}_2O_4$		3.22 O	2.33	0.004	9.0 Zn	3.81	0.009
sphalerite	ZnS						
					9.1 s	4.46	0.01
aqueous	Zn^{2+}	5.7 O	2.07	0.010			
Zn oxalate	$ZnC_2O_4 \cdot 2H_2O$	6.4 O	2.07	0.009 0	4.3 C	2.80	0.005
lithiophorite		6.4 O	2.02	0.009	4.7 Al	2.95	0.004
$(Li,Al)(Mn)O_2(OH)_2$							
Zn-sorbed soil		6.9 O	2.08	0.008	0.6 Al	2.99	0.001
Zn-sorbed HIM		5.7 O	2.05	0.010	3.8 Al	3.05	0.006
Zn-copr. HIM		6.3 O	2.07	0.009	6.0 Al	3.06	0.006
Zn-Al LDH		6.3	2.07	0.009	3.9 Zn	3.10	0.008
$[Zn_{1-x}Al_x(OH)_2]+(x)Cl \cdot mH_2O$		6.5 O	2.01	0.013	6.3 Mn	3.42	0.007
chalcophanite							
$(Zn,Fe,Mn)^{II}Mn_3{}^{IV}O_7 \cdot 3H_2O$							
Zn sulfate	$ZnSO_4 \cdot 2H_2O$	5.6 O	2.07	0.006			

[a] Coordination number, [b]Radial distance, [c]Debye-Waller factor

Surprisingly, pyrite was the only Fe sulphide present in the sediment. Concentrations of **Cu** and **Ni** were too low to allow good X-ray absorption spectra to be obtained. It was demonstrated that HCl-extraction dissolved also other sedimentary metal phases. Although Pb dissolved rapidly after 24 h in HCl, it was not present as sulphide, as evidenced by EXAFS and a geochemical modelling of the porewater suggested its association with carbonates (very soluble in strong acid) or sorption as an oxygen-ligated surface complex (also desorbing in strong acid). Like Cu and Ni, also **Cr** was poorly extracted in HCl. XAS analysis again indicated association with oxides. Table 5.8 just illustrates the type of structural information we obtain by XAFS spectroscopy necessary to determine the most dominant species for Zn (here reference compounds), as an example, in a sediment (or soil) sample.

In a similar study, also Cooper & Morse 1998 (see also section 5.4.3.3) showed that Cu and Ni sulphides (e. g. NiS, NiS_2, Ni_3S_2, CuS, Cu_2S) are poorly extracted by cold acid (1-31 % after 1 h in 6 M HCl). Without spectroscopic evidence, it remains uncertain whether Ni and Cu are present as sulphides in the sediment. Like Cr, also Ni seems most likely to be associated with weathering products (such as secondary clay minerals).

The vertical distribution of AVS in the sediment showed a significant concentration increase with depth. However, the performance of a dissolution time series uncovered that at 30 cm depth all AVS dissolved already after 30 min, whereas metal dissolution was not complete at the same time, even after 24 h. Measuring sediment charcteristics showed that dissolved O_2 was removed within the first 2-10 mm below the sediment surface, and that dissolved sulphide below a depth of 30-40 mm occurred throughout the sediment core. Based on porewater concentrations the authors supposed that dissolved sulphide (HS^-) accounts for most if not all AVS extracted from the sediment. Also, the obtained spectroscopic data could not confirm that FeS(s) is the primary contributor to AVS in these sediments as commonly assumed in the ∑SEM/AVS measurement (O’Day et al. 2000).

The observation that metals in reduced anoxic sediments may occur both as sulphide and oxide solid phases is still rather controversial among experts and has implications for establishing sediment quality values (see chapter 8). The study of O’Day et al. (2000) at least shows that the ∑SEM/AVS concept was indeed only valid for Cd and to some extent for **Zn**. Consequently, oxidative dissolution of metal sulphides would only affect these two metals. Other metals, like **Cr** and Pb, and also **Ni** and **Cu**, seem to bind more to oxide and silicate surfaces, maybe as inner-sphere complexes and precipitates of carbonate and oxyhydroxide, which are pH-dependent. Partitioning of these metals seems more affected by early diagenetic changes in pH and mineral substrate stability (aging!) occuring within the uppermost part of the sediment profile (see also section 5.5.6).

When it comes to sediment toxicity, the ∑SEM/AVS ratios of 2.7-5.25, measured by O’Day et al (2000) did not support the toxicity prediction they suggest, mainly because porewater metal concentrations at the sediment-water interface were too low. This was in contrast to deeper sediment layers, where a 100% toxicity was observed at ∑SEM/AVS = 0.54, probably due to high AVS (sulphide!) values. However, further porewater analyses showed that the observed invertebrate toxicity possibly was attributable to ammonia and low oxygen levels, although this was not easy to verify due to variable organism responses and the geochemical heterogeneity of the sediment. Based on the toxicity and spectroscopic data, the authors conclude that the ∑SEM/AVS threshold (<1) is not a good criterion for predicting acute organism toxicity in aquatic sediments, a fact which has been already acknowledged by the US EPA in the mean time (see ref. therein). In particular, the obtained spectroscopic results indicate that the equilibrium partitioning given by $Me^{2+}(aq) + FeS(s) = MeS(s) + Fe^{2+}(aq)$ as rationale for the ∑SEM/AVS concept may be valid only for Cd, partially for Zn, but not for Pb, Cu and Ni in these estuary sediments. So, at its best measuring AVS may be a useful indicator for Cd and Zn availability in reduced sediments, but not a general measure of bioavailability for trace metals in a variety of geochemcial environments as proposed.

Organic and inorganic particles (e. g. humics, polysaccharides, microorganisms, Fe and Mn oxides/hydroxides, clays) among others are supposed to mediate the cycling of essential and toxic metals in aquatic systems. ’**T**ransmission **E**lectron **M**icroscopy’ (TEM) coupled with ’**E**nergy **D**ispersive **S**pectroscopy’ (EDS) has been successfully applied before in speciation studies to provide informations on size, morphology, crystallinity and composition of sediment particles, and on possible physical and biological associations. Previous artefacts produced through the dehydration process during sample preparation leading to shrinkage and aggregation of particles can now be overcome by utilizing a hydrophilic resin (‘Nanoplast’) to embed the sample. Web et al. (2000) examined the direct association of **zinc** in a highly contaminated lake sediment with up to 30 % Zn d.w. at the individual particle scale by TEM/EDS analysis, and the influence of biological processes on Zn distribution.

Electron microscopy revealed the existence of different *colloidal particles* in *more oxic* lake sediments, including bacteria, small algae, organic fibrils and matrices, clay minerals, Fe hydroxides, Fe-Zn-phosphates, biogenic silica and diatoms, sulfidic minerals, and to a minor extent carbonates of Ca and Mg. *Zn-bearing colloids* were often strongly associated with or entrained into biological structures. They seem intimately associated with algal cells, whose organic-rich outer surface may act as a nucleation site for particle formation. In these particles Zn may coprecipitate or coentrain at the observed high concentration, and not only become bound by surface adsorption. Other Zn-containing particles included Fe-Zn-oxihydroxides and to a lesser extent Zn sorbed to Fe-rich clay minerals. Also particles in the water column (with up to 300 µM Zn) consisted mainly of diatom frustules, clay minerals and organic matrices. In general, Zn was at most associated with Fe-P-rich particles or sorbed to Fe/Mn-rich precipitates on bacterial cell walls, but not on clay minerals, demonstrating the clear relationship between biological structures and Zn partitioning. In contrast, in a *more anoxic* lake sediment sample, particulate Zn was associated with small rounded globules (45-210 nm size) and mineralized layers, together with high sulfur concentrations suggesting a diagenetic formation of ZnS.

Speculating on the origin of the observed Fe-P-Zn-bearing particles in the sediment, Webb et al. (2000) resume that they may be caused by biological processes leading to phosphorus precipitation on the cell wall. Indeed, TEM/EDS anaylsis showed that these particles are associated with *biological cells* and surrounded by *extracellular structures*, and may act as Zn carriers in the lake, but also as catalyst site for particle formation. The authors claim more research to elucidate processes occuring at the cell wall level and leading to the formation of colloidal metal-bearing particles. They also refer their results to the current literature, from which we know that *fibrils* (extracellular polymers) on *bacterial surfaces* provide appropriate nucleation sites for Fe and Mn oxides, that bacterial cell walls can remove and accumulate metals from dilute solutions, that generation of mineralized scales (Ca, Si) and wall parts of colloidal dimensions induced by internal metabolic processes in microalgae occur, and that Fe oxyhydroxide coatings on cell walls and extracellular fibrils strongly can accumulate **Cu** from porewater. All these studies prove the importance of the microscale to identify relevant contaminant-bearing phases in aquatic ecosystems, and of the *biological templates* as important sites for contaminant transformation. However, one short-cut of the used approach was the used X-ray detector, which was only sensitive to relatively high elemental concentrations (> 0.1 %) and hence does not cover particles with smaller metal concentrations, but sufficiently abundant to make up a significant fraction of the total metal concentration. Complementary techniques, like XAS would help to overcome this limitation (more speciation studies using spectroscopic analysis are presented in sections 5.5.4, 5.5.5, and 5.5.6, below).

5.4.6 Bioturbation, resuspension and bioirrigation

Oxidation of sediment-associated sulphides during episodes of sediment resuspension, or by introducing oxygen-rich water into non-surficial sediment layers, e.g. by burrowing organisms (bioirrigation), is supposed to be a major mechanism of metal release from sediments to the overlying water. Sediment mixing by benthic animals (bioturbation) can additionally disrupt metal sulphide binding in sediments, while released metals may precipitate again with Fe or Mn hydroxides, or become complexed by organic matter. In fact, new experimental results show that *iron monosulphides may act as a buffer for the initial oxidation of associated trace metal sulphides during exposure of anoxic sediments to oxic water, as in the case of bioturbation. New research suggests that biological kinetics (i.e. metal uptake and accumulation processes) are much faster than chemical kinetics (i.e. metal release by desorption from the sediment). Sediment-burrowing organisms tend to accumulate more metals than are initially available in the dissolved phase, indicating that additional metals have to be delivered from various solid phases to the dissolved metal pool, probably due to sulphide oxidation by burrowing, desorption from surfaces or dissociation of precipitated complexes.*

One assumption from what has been presented in section 5.4.4 is that sufide minerals may act as a permanent sink for many trace metals. However, Cooper and Morse (1996) (cited in Cooper and Morse, 1998) demonstrated that up to 90 % of *estuarine pyrite* can oxidize within 1 day of exposure to oxic water and release associated trace metals into the “reactive” HCl-soluble pool indicating that *oxidative dissolution of sulphides* (e. g. by bioturbation, storm resuspension, or dredging operations) could in contrast act as an important source for metal fluxes into overlying waters.

Bioturbation includes filter feeders injecting oxygenated water into deeper sediment layers, and surface dwellers, which regularly resuspend the top surface layer. The oxidation of sulphide phases in anoxic sediments due to *resuspension* or by introducing overlying oxic water at depths, e. g. by burrowing organisms (*bioirrigation*) is supposed to be a major source of metals released to the overlying water, but has not been considered yet as relevant for bioavailability and toxicity studies. Bioirrigation rates as high as 750 ml/h have been reported to occur in natural sediments (Foster-Smith (1978) cited in Simpson et al. 1998). In this context, it has been asked, if bioturbation can significantly disrupt metal sulphide binding in sediments. Or, if FeS and MnS can buffer the effects of bioirrigation at least for a certain time? Oxidized sulfur species (like SO_4 and S^0) and released metals may subsequently become quickly scavengened again by Fe or Mn hydroxides or complexed by organic ligands in the sediment.

To find an answer to these questions, Simpson et al. (1998) exposed model substances (metal sulfides) to aerated water to simulate the effect of short-term *resuspension* on solid metal speciation of contaminated anoxic estuarine sediments. As one result, FeS and MnS appeared as labile and rapidly oxidizable phases in contrast to CdS, **CuS**, PbS and **ZnS** model phases, which were kinetically stable over periods of several hours and, therefore, not equally affected by resuspension. The observed rapid decrease of AVS (from 119.7 to 20 µmol/g) upon resuspension observed in natural sediments was consequently related to the oxidation of iron monosulphide, but was slower than observed for the FeS model compound. After prolonged resuspension, AVS dropped to significantly lower levels (10 µmol/g) than SEM (∑Cd, Cu, Ni, Pb, Zn dissolved in 1 N HCl) indicating that also part of the trace metal sulphides have been oxidized during ongoing resuspension. Also *total reducible sulphide (TRS)*, which includes pyritic S, AVS, organic and colloidal S, decreased from 720 to 600 µmol/g during an 8h-resuspension period, but did not give evidence that pyrite oxidation was significant (see also the study by Otero and Marcias 2002, below). Actually only SEM-**Cu** increased during an 8h-resuspension from originally 0.1 to about 2 µmol/g, while all other metal-SEMs remained constant. But it was shown that the observed increasing SEM-Cu concentrations turned out to be an *artefact* of the used SEM/AVS procedure: during HCl-oxidation, $Fe(OH)_3$ was produced in solution and dissolved again upon acidification to Fe^{3+}, which subsequently oxidized CuS in the sediment. During resuspension, Fe^{2+} was released through the rapid dissolution of FeS to FeII and SO_4. Subsequent oxidation of Fe(II) to Fe(III) occured in turn slowly (minutes to hours at pH 7-8). Summarized, it was shown that dissolved Fe(III) could act as an effective oxidant for Cu sulphide first after acidification of the sample in the SEM/AVS extraction step. Based on their experimental results, Simpson et al. (1998) concluded that in natural sediments, iron monosulphides may act as buffer for the initial oxidation of associated trace metal sulphides during exposure of anoxic sediments to oxic water, for example in the course of bioturbation.

The *contradicting behaviour* of natural sediment sulphides and model sulphides may point out to the possibility that a significant fraction of the trace metal sulphides are not present as AVS but as pyritic fractions in the sediment. *Trace metal pyritization* is known to be an important sink for metals in marine anoxic sediments. Morse (ref. given in Simpson et al. 1998) observed that oxidative release of metals from authigenic pyrite was greater than the extent of pyrite oxidation. As a matter of fact, metals adsorbed to the pyrite surface may be acid-soluble and so observed in the SEM-fraction, although no sulphide would be released. In this context, also metal binding on particulate organosulfur phases is still poorly understood. In as far microbial-catalysed oxidation may contribute to the observed discrepancy observed between AVS in natural sediments and model sulphides, remains to demonstrate.

For this reason, Simpson et al. (2000a) question the SEM/AVS theoretical assumptions that (1) only monosulphide phases are extracted and that for every mole sulphide a corresponding mole of metal is measured in the SEM fraction. And (2) the fact that discrete CuS and NiS proved hardly soluble in 1 M HCl renders only little theoretical basis for the interpretation of sediment toxicity from SEM/AVS data at least for these particular metals. The authors further argue that (3) the observation that Fe^{3+} oxidizes CuS during SEM extraction without a corresponding increase in AVS, which again underlines the rather conservative nature of the AVS approach in assessing the bioavailable part of sediment-bound metals. That stoichiometrically different Cu sulphides may be extracted to different extents seems to make SEM/AVS measurements even more difficult for a proper data interpretation. In fact, the observation that Cu_2S may be more oxidized than CuS, can be explained by the stoichiometrically smaller amount of sulphide in Cu_2S to be available for oxidation. Another source of error (according to the authors) when measuring AVS (with or without Fe) may be its oxidation to SO_4 or S^0 rather than due to its dissociation to form H_2S, which is finally measured as AVS.

Recent laboratory resuspension experiments with defined metal sulphides showed that ZnS, PbS and CdS were resistant against oxidation in seawater (Simpson et al. 2000a). In contrast, AVS in highly anoxic and metal contaminated field sediments was rapidly oxidized with < 6 % remaining after 24 h, despite high particulate Zn and Pb contents. For this reason, Simpson et al. (2000a) concluded that these metals may occur largely (73-95 %) as non-sulphides. By subsequently adding soluble ionic Zn to the sediment, ZnS was quantitatively formed suggesting a rapid formation of *sulphide coatings* that protect particles against further *sulfidization* (see section 5.4.4). Table 5.9 shows that (beside Zn powder) added solid Zn and Pb phases dissolved completely during the AVS extraction with recoveries of 88-107%. Upon resuspension in seawater, the AVS concentration of sediments spiked with $ZnCl_2$ and $ZnCO_3$ reached a plateau corresponding to about 75% of the added solid phase, while other phases were even less (10-31%). AVS/SEM analysis 1 and 3 months later did not show any consistent changes, suggesting that a significant portion of metals entering aquatic systems as particulates may become *sulfidized* for a long time (years). From this, it seems that a rapid formation of *insoluble sulphide coatings* on the particle surface may occur and protect them against further reaction (sulfidization).

Table 5.9. Reactivity of added solid phase metal constituents (M) in Iron Cove sediment[a] and final metal sulphide formation upon sediment resuspension in seawater (from Simpson et al., 2000a)

Solid added	added M (µmol/g)	recovered M (%)[b]	AVS (µmol/g)		metal sulphide formation (%)
			0 h[c]	24 h	
nothing	0	233 (11)	11 (5)		
Zn powder	70	69 (10)	231 (36)	21 (18)	15
ZnO	70	101 (7)	225 (20)	20 (9)	
13					
$ZnCl_2$	70	100 (5)	242 (35)	64 (8)	76
$ZnCO_3$	120	107 (8)	240 (17)	104 (7)	75
PbO	70	97 (10)	211 (19)	18 (4)	10
$PbCl_2$	70	88 (8)	210 (41)	33 (6)	31

[a]Standard deviations are given in parentheses, [b]percent of added solid-phase metal recovered as SEM (Zn or Pb), [c]sediment resuspension time

There is increasing evidence today saying that reactive metal sulphides may not play the role in the speciation of metals entering anoxic sediments, as suggested so far by the AVS concept. According to this approach, sediments with an excess of reactive sulphide will have very low (nontoxic) dissolved metal levels in the porewater, due to the low solubility of metal sulphides. The molar excess of acid volatile sulphide (AVS) (mainly FeS) over simultaneously extracted metals (SEM) is consequently used to predict the absence of metal bioavailability and toxicity to benthic biota. It is further supposed that AVS extraction by 1 M HCl (45 min) extracted H_2S, amorphous FeS, mackinawite (FeS_{1-x}), some greigite (Fe_3S_4) but not pyrite. In their experiments, Simpson et al. (2000a) could show that, although CdS, PbS and **ZnS** were completely extracted, dissolution of **NiS** and **CuS** was limited. Significant *artefactual oxidation* by released Fe^{3+} was supposed to occur during AVS extraction, most likely due to reactions following acid addition (see above). Unlike for CdS and ZnS, PbS dissolved only slowly during AVS extraction allowing time for Fe^{3+} to act as an oxidant, which may explain the lower recoveries of PbS in the resuspension experiment with model sulphides, in contrast to CdS and ZnS. Adding Zn^{2+} or ZnS to the sediment rapidly decreased AVS concentrations upon resuspension indicating that added Zn reacted with sediment AVS and that the formed ZnS was resistant to oxidation. Resuspending the sediment with oxic seawater resulted in AVS values well below SEM. Considering the rapid formation of metal sulphides and their resistance to oxidation (upon resuspension), the authors hypothesize for Pb and **Zn** that these metals were not present as discrete sulphides in the sediment. The possibility that metals entering sediments in a metallic form, or as oxides or salts, become sulfidized, was confirmed by the performed experiments showing that the formed sulphide coatings can prevent further sulfidization of these solid metal forms. The formation of surface coatings of Fe(II) sulphide on amorphous Fe (III) oxyhydroxide particles was already suggested in previous studies (see Cooper and Morse 1998, Simpson et al. 1998).

Vink (2002) developed a so-called '**S**ediment **O**r **F**auna **I**ncubation **E**xperimental system' (SOFIE) to analyse the chemical speciation of metals in porewaters of natural undisturbed sediments at the sediment-water interface, while simultaneously conducting exposure tests with test organisms causing significant bioturbation. It was shown that metals competing for binding opportunities between reactive sulphides and dissolved organic matter explain the observed high dissolved concentrations in sulfidic zones. However immediately after introducing a *sediment dweller* (here the oligochaete *Limnodrilus*), equilibrium concentrations at once changed (see Table 5.10). *Limnodrilus* lives in *burrows and irrigates* the sediment with overlying oxic surface water. The author observed pronounced effects after colonization of the model sediment by the oligochaete: the redox potential (E_h) rised significantly and pH increased by half a unit, sulphides became oxidized and phosphate released. Also, macronutrients are mobilized and taken up at the same time by benthic organisms for their growth and metabolism. But, these organisms also accumulate and eliminate metals. From this one may conclude that the newly established dynamic state of the sediment-water system confirms that *biological kinetics* (i. e. uptake and accumulation processes) are much faster than *chemical kinetics* (i. e. release by desorption from the sediment). A continued steady flux was observed only for As (not shown) and **Ni** from sediment to porewater. The obtained results in Table 5.10 show that relatively large fractions of free ion activities occur in the overlying oxic surface water. These *free metal fractions* may increase after some days when organisms are introduced and decline again after increasing uptake of metals by the test organisms. One has to keep in mind that high free metal ion activities occurring in the overlying oxic water do not necessarily mean high total concentrations.

Table 5.10. Free metal ion activities as percentage of total dissolved concentration at equilibrium, and 7 and 21 days after introducing test organisms (Limnodrillus spp.) (from Vink, 2002)

free metal activitiy (% of total dissolved concentration)				
Metal	distance water-sediment (mm)	steady-state	7 days after introducingLimnodrillus	21 day after introducing Limnodrillus
Cd	+5	29	48	<1
	-5	<1	1	40
	-10	<1	26	12
	-15	<1	<1	29
	-25	<1	nd*	17
	-30	<1	<1	20
	-35	<1	13	<1
	-40	nd	<1	<1
Cu	+5	4	13	1
	-5	8	5	<1
	-10	4	10	<1
	-15	<1	1	<1
	-25	<1	nd	<1
	-30	<1	<1	<1
	-35	<1	<1	<1
	-40	nd	<1	<1
Ni	+5	29	26	18
	-5	1	23	5
	-10	<1	8	1
	-15	<1	19	<1
	-25	<1	nd	<1
	-30	<1	<1	<1
	-35	<1	<1	<1
	-40	nd	<1	<1
Pb	+5	1	34	1
	-5	1	1	1
	-10	<1	15	1
	-15	<1	1	<1
	-25	1	nd	<1
	-30	5	1	<1
	-35	1	2	<1
	-40	nd	1	<1
Zn	+5	38	44	32
	-5	55	35	28
	-10	1	47	1
	-15	36	1	1
	-25	34	nd	5
	-30	4	1	1
	-35	5	34	1
	-40	nd	1	1

*nd = not determined due to failure of the micro-seized ion-exchange columns

Mass calculations showed that *Limnodrilus* consumed considerably more metals than initially available in the dissolved phase: for Cd 18 times, for **Cu** 4.4, for **Ni** 1.3, for Pb 122 and for **Zn** 10 times more. From this it becomes evident that more metals have to be delivered from various solid phases to the dissolved metal pool, probably due to sulphide oxidation by burrowing, desorption from surfaces or dissociation of precipitated ligands. However body concentrations decreased again for all metals after 11 days (except for As). Statistical analysis of the data showed that the uptake of Cd, Cu, Ni and Zn was indeed related to the free metal ion activity in the overlying water. This was not surprising as *burrowing organisms* exchange the water in their burrows with overlying water, which was characterized by a higher free metal ion pool than the porewater.

In summary, the studies showed that burrowing organisms will significantly affect metal speciation. Introducing organisms into the sediment will result in the release of metals mainly due to the dissolution of Fe and Mn oxyhydroxides and oxidation of sulphide precipitates. Metals are simultaneously released and taken up again producing antagonistic processes, with rising body concentrations and a decline of the free metal ion activity. In contrast to Cd, Cu, Ni and Zn, As and Pb uptake was best related with total dissolved porewater concentrations. Constructing similar generic models that can transfer chemical information to a biological response should have high future research priority. In this context the FIAM concept, as described by Vink (2002), is a significant step forward (see the following section 5.4.7.1, and chapter 6).

That the resuspension of contaminated sediments may indeed constitute a potential source of toxic compounds, including trace metals, for aquatic ecosystems, was recently also demonstrated by Geffard et al. (2002). The authors examined the release, bioavailability, bioaccumulation and toxicity of PAH and trace metals in resuspended contaminated coastal sediments, and possible effects on the embryogenesis and larval growth of *Crassostrea gigas*. Test organisms were either directly exposed to elutriates or fed on algae (*Isochrysis galbana*) contaminated with these elutriates. Both toxic effects and bioaccumulation were more pronounced when the larvae were directly exposed to sediment elutriates. Based on these findings, the authors speculate that a fraction of the studied contaminants (metals and PAHs), adsorbed on and released during resuspension (extraction) from the sediment, was bioavailable and subsequently bioaccumulated by the test larvae.

5.4.7 Metal-ligand interactions

The 'free ion activitiy model' (FIAM) assumes that the uncomplexed free metal activity rather than total or dissolved concentrations reflects the portion of metal that is directly bioavailable and, thus, is responsible for possible biological effects. Predicting the uptake of metals from complex natural systems by the FIAM-model is based on the assumptions that mass transfer is not flux-determining, complex association and dissociation reactions are at equilibrium (meaning that bulk and surface activities for all metals are the same), and that the free ion activity controls reactions with surface sites on organisms and, thus, metal bioavailability. However, in natural systems the limiting flux of free ions is much larger than the real uptake into organisms. From recent studies it seems that bio-uptake itself may provoke dissociation of complexes and therefore increase the supply of free metals, which is finally defined by the rate of diffusion of free metal ions, or of different labile metal complexes.

'Surface complexation models' (SCMs) describe the adsorption of metals on pure minerals, soils or sediments. In these models it is assumed, e.g. that (1) mineral surfaces are composed of specific functional groups reacting with dissolved metals to form surface species, and that (2) the electrical charge at the surface is determined by the chemical reaction of the particular mineral functional groups.

There are various methods available today to assess the surface site density of single minerals. Howevere, for complex mineral assemblages, the so-called "reactive" specific surface area is measured. From this, one can quantify the concentration of functional groups that participate in surface complexation equilibria, by measuring site densities (per unit surface area) of single minerals. Recent research suggests that most adsorption data can be modelled by assuming two non-equivalent surface binding sites, i. e. a "strong" and a "weak" site (two-site models). As a whole, all these experimental and modelling techniques are still not so far developed to reliably quantify the relative abundances of various surface functional groups in complex mineral systems, or to identify surface functional groups that dominate adsorptive interactions.

It has always been a challenge for environmental scientists, like chemists or biologists, to assess the behaviour of trace metals in natural systems solely based on either purely physicochemical or biological characteristics. For this reason, there are increasing attempts underway now to bridge both aspects by combining biological and chemical standardized methods and by integrated modelling of both types of data (see in particular the following chapter 6).

How complex the relationship between metal speciation and bioavailability can be, was recently shown by Eimers et al. (2002). Stable Cd isotope ratios in sediment and water were measured and compared to the ratios measured in asselids to examine whether Cd partitioning could predict *metal bioaccumulation*. Alhtough it was shown that Cd concentrations in the overlying water determined accumulation in *Assellus*, it was also uncovered that increasing sediment organic matter decreases the concentration of Cd in the overlying water and so bioaccumulation of this metal.

In particular the comparative importance of geochemical, metal-specific and organisms-specific influences on metal bioavailability has not been quantitatively considered yet. Aquatic sediments constitute an important food source for many deposit- and suspension feeding animals. To assess the potential impact of sediment contamination to aquatic ecosystems, we have to assess the degree to which sediment-bound metals are *assimilated* into animal tissues and the mechanisms controlling their bioavailability. As seen before, caution should be employed in comparing experimental results derived from distinct single-components in the laboratory, to data obtained from the field. Generalizations are possible within some types of ecosystems, but difficult to develop across metals or ecosystems. So far, bioavailability studies directly related to geochemical species are still rare. Despite an obvious need, surprisingly little research has been devoted during the last years to attempts to link the concept of bioavailability of metals (their uptake, assimilation and accumulation in organisms) to their chemical speciation.

In the following, we will present some examples to illustrate some of the most recently developed concepts to measure, describe and explain the interaction between metal speciation and bioavailability.

5.4.7.1 Free ion activity model (FIAM)

Concerning a quantitative determination of the portion of metals, which is really bioavailable, the *'free ion activitiy model'* (FIAM) seems to point into the right direction, as supposed by many scientists today. It assumes that the uncomplexed free metal activity rather than total or dissolved concentrations is decisive for possible biological effects in organisms exposed to metal-contaminated sediments. According to Vink (2002), however, evidence for the success of FIAM is still rather limited and its full applicability to natural waters remains still to be demonstrated, in particular due to the presence of natural dissolved organic matter (DOM). For this reason, future progress is expected from methods able to measure free metal ion activities also in the presence of *naturally occuring organic ligands*.

We know that DOM decreases metal toxicity due to decreasing the free ion activity, although total dissolved metal fractions may increase.We also know that the free Me^{2+} activity increases in response to total metal content and increased solution acidity, which supports the the assumption that the free metal activity is controlled by complexation-adsorption rather than mineral solubility equilibria. Metal ion activity calculations based on chemical equilibrium reactions also seem limited due to the lack of *kinetic considerations*. In-situ techniques like ion-selective electrodes, or DGT (see section 5.5.3), dialysis or millipeeper methods (for porewater recovery), may overestimate ion activities as they also respond to and interfer with other elements (like sulphides, metals, DOM), or they are not able to monitor concentration shifts during organisms exposure.

In a more theoretical contribution, Leeuwen (1999) states that predicting biouptake of metals from complex systems by the ‘free ion activity model’ is limited to conditions where *mass transfer* is not flux-determining. One major assumption of FIAM is namely that *transport* of a metal in solution is fast compared to metal *uptake*, so that the latter can be considered as the *rate-determining step*. Also complex association and dissociation reactions are supposed to be at equilibrium, meaning that bulk and surface activities for all metals are the same. For this reason it is supposed that the free ion activity controls reactions with surface sites on the organisms and consequently governs bioavailability. For complex natural systems the limiting flux of free ions should be much larger than the real uptake flux to organisms. Here, one may ask whether an organism will be satiesfied with the very small metal flux or if biouptake itself will *provoke the dissociation of complexes* and so *increase the supply of free metals*. For a wide range of concentrations, the relation between biouptake and bulk concentrations has proved to be non-linear, whereas for the transport rate it is. There are systems where mass transport may not be negligible with respect to biouptake, e. g. where complex dissociating conditions contribute to the transfer of metals to the phytoplankton cell surface suggesting radial diffusion and complexes with diffusion coefficients equal to those of free metals.

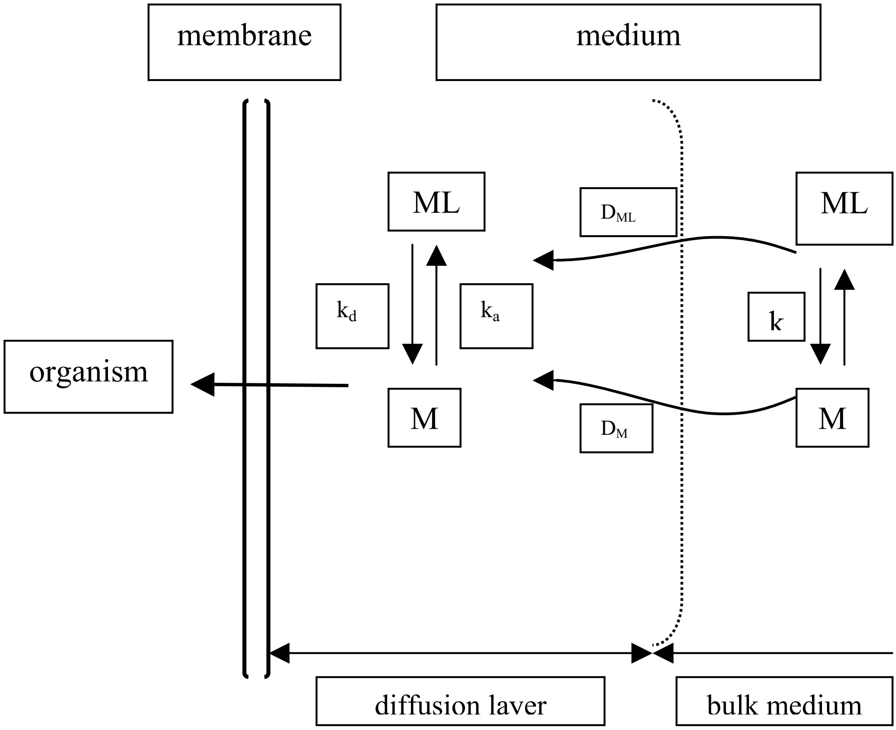

Figure 5.1. Schematic representation of the role of complexation reactions in the medium of a metal-uptaking organism. Reprinted with permission from Leuuwen, 1999 (American Chemical Society)

The overall scheme of the processes of biouptake of the free metal ion (M) according to Leuuwen (1999) is depicted in Figure 5.1, including the diffusional transport of M with the diffusion coefficient D_M, the interconversion between M and ML (complexed metal ion) governed by the rate constants k_a and k_d, and the possibly resulting coupled diffusion of ML (with diffusion coefficient D_{ML}). Since many environmentally relevant complexes diffuse much slower than M, the distinction between D_M and D_{ML} is important. The actual biouptake follows a Langmuirian-type adsorption of M at the outer cell wall and a subsequent first-order rate-limiting internalization step. The supply of free metal ions (M) is finally governed by the diffusion of free metal or complexed diffusion of the different labile metal species (ML).

5.4.7.2 Surface complexation

'Surface complexation models' (SCMs) are increasingly used today to describe the adsorption of metals on pure minerals, and partly on natural aquatic sediments. Three main types of SCMs have been developed, i. e. the *'capacitance model'* (CCM), the *'diffuse layer model'* (DLM), and the *'triple layer model'* (TLM).

According to Davis et al. (1998) there are four tenets common to all surface complexation models:

(1) the mineral surface is composed of specific functional groups that react with dissolved solutes to form surface species (i. e. coordinative complexes or ion pairs) analoguous to the complexation in homogeneous solutions
(2) equilibria of surface complexation (SC) and surface acidity reactions can be described by mass action equations
(3) binding constants can be related to thermodynamic constants by the activity constants of the surface species, and
(4) the electrical charge at the surface is determined by the chemical reaction of the mineral functional groups

Most work on SC has been done on the adsorption of ions by a variety of discrete mineral surfaces. Applying it to the adsorption of ions on soils and sediments is still rather rare due to the complexity of these natural systems. For example, the adsorption experiments by Davis et al. (1998) with **Zn^{2+}** showed a comparably smaller pH range of the *"adsorption edge"* on quartz powder than on natural sediments, probably due to the greater heterogeneity of surface functional groups existing on complex mineral surfaces in soils and sediments (including for example organo- and phosphato ligands). Davis et al. (1998) also point out to the fact that the surface properties of minerals in soil and subsurface environments are much altered by accumulating poorly crystalline Fe and Al oxyhydroxides and silicates. Subsequent early diagenesis together with bacterial interactions may leach these surface layers and consequently form so-called mixed-layer clays and/or cause the deposition of extremely fine-grained high-surface area precipitates. These processes will handicap a proper use of the SC modelling, as some assumptions in the development of SCMs for pure single minerals may not be valid any more for complex mineral assemblages (like soils or sediments). In many if not most cases, surface properties of sediments are determined by secondary minerals or grain coatings although constituting a minor amount/fraction of the total sediment mass. As an example, the authors refer to a study (ref. given), in which surfaces of silica, rutile, ferrihydrite and goethite became enriched with Al when mixed with poorly crystalline alumina or with a dissolved Al-containing solution, resulting in a kind of surface enrichment, which can completely alter the surface properties of minerals. The authors also resume that the formation of Al- and Fe-rich surface coatings observed in their experiment, was the cause why quartz powder surfaces proved to be less reactive than natural sediment surfaces.

A variety of methods is now available to assess the *surface site density* of single minerals, but experimental interferences still hamper their use for natural solids. To determine surface site densities for SC modelling of complex mineral assemblages, Davis et al. (1998) suppose to measure the *"reactive" specific surface area* (e. g. by the 'BET' method; ref. given). Once the specific surface area is known, one can quantify the concentration of functional groups that participate in SC equilibria from measuring site densities (per unit surface area) of single minerals, which in turn are available for many discrete mineral phases from the relevant literature.

There are two modelling approaches supposed to provide first predictions of the adsorption of trace metals by natural sediments. For the *'component additivity'* (CA) approach, the surface area of each mineral present at the surface is multiplied by the site density established for that mineral. For the *'generalized composite'* (GC) approach, the recommended value for site density is 3.84 µmol of surface sites per m^2 of surface area, which is consistent with the value recommended for ferrihydrite given in the literature (ref. given by Davis et al., 1998).

To account and correct for *Coulombic effects* characteristic in mass action equations, several SC models and various approaches have been proposed according to Davis et al. (1998). To give an example, the *diffuse double-layer model* (DDL) suggests that one proton is released per **Zn^{2+}** adsorbed at the mineral surface (in eq. 5.10 and 5.11), but that the formed surface complex promotes additional proton release through Coulombic repulsion, according:

$$Zn^{2+} + {\equiv}SOH \overset{k_{Zn}}{\leftrightarrow} {\equiv}SOZn^{+} + H^{+} \qquad (5.10)$$

("one site – one proton model")

$$Zn^{2+} + {\equiv}SOH + H2O \overset{k_{ZnOH}}{\leftrightarrow} {\equiv}SOZnOH + 2H^{+} \qquad (5.11)$$

("one site – two proton model")

(≡SOH = functional surface group, k_{Zn} = apparent stability constant for mass action equation)

In fact, correcting for these electrostatic effects, calculation with the DDL model suggests that 1.7-2.0 protons are released per Zn^{2+} adsorbed on ferrihydrite depending on pH and ionic strength.

In contrast to this, the *'non-electrostatic model'* (NEM) is more straightforward and assumes surface equilibria strictly as purely chemical reactions without correcting for electrostatic effects. The pH-dependent Coulombic energy is simply included into the resulting binding constant. As one consequence, derived mass action equations that describe ion adsorption do not provide an accurate stoichiometry of the involved reactions at the molecular scale. But since it is difficult to assess the actual surface site density of complex mineral mixtures (like soils or sediments), it is usually not possible to realize a proper *electrostatic correction* necessary for developing more complex SC models, like DDL or TLM (triple-layer model).

In this context, Davis et al. (1998) also point to the fact that the *surface charge of minerals in natural waters* is different from simple electrolyte solutions. As an example, the formation of organic coatings and the adsorption of major ions (like Ca^{2+}, Mg^{2+}, SO_4^{2-}, silicate) change the *point-of-zero-charge* (pH_{PZC}) and *isoelectric point* (pH_{IEP}) of minerals. In addition, their obtained experimental data proved that the pH dependence of Zn^{2+} adsorption on natural sediments is less steep than on hydrous oxides (as expected). The data suggest that Zn^{2+} adsorption on natural sediments fits best with eq. 10, while adsorption by quartz fits best with eq. 11. Also, pH dependence of Zn^{2+} adsorption was well described with only one surface reaction. From this, it may be concluded that most adsorption data can be usually modelled by assuming two non-equivalent surface sites, i. e. a "strong" and "weak" binding site.

However, according to the 'NEM' model, the average *'free energy'* of the complexation of $\mathbf{Zn^{2+}}$ with surface sites on a natural sediment is characterised by assuming that all surface sites are chemically equivalent (one-site model). Experimental techniques to quantify the relative abundances of various surface functional groups in complex mineral systems, or to identify surface functional groups that dominate adsorptive interactions, are still not well developed. The authors refer to recent studies, where *'SIMS'* and *'Auger spectroscopy'*, and aqueous extractions revealed that sediment grains are covered with Fe-, Al- and partly Mn-rich coatings probably derived from the weathering of feldspars and Fe-bearing minerals. Preliminary calculations done for goethite suggest that *surface hydroxyls* were not present in sufficient abundance to solely explain the adsoprtion of Zn^{2+} by the sediment. In the authors' opinion, the 'GC' approach seems therefor more preferable to other empirical approaches, like establishing distribution coefficients (k_d) or adsorption isotherms.

The work of Davis et al. (1998) indicate that both SC modelling approaches presented here (i. e. CA and GC) can be successfully applied to model adsorption of metal ions by soils and sediments. But the authors claim, future research should focus more on a better characterization of the composition of *sediment surface coatings* at molecular levels as well as on the utility of specialized aqueous extractions as a means to quantify *surface functional groups*.

In this context, future research should also address the questions of how dissolved or adsorbed ligands control the bioavailability of metals to organisms (see chapter 6), and how to reliably quantify the thermodynamic characteristics of adsorbed and porewater metal binding ligands.

Mouvet and Bourg (1983) (cited in Wen et al. 1998) were the first, who reported that adsorption constants of metals are directly proportional to their first hydrolysis constants. Based on acid-base titrations and adsorption experiments, Fu and Allen (1992) (also cited in Wen et al. 1998) subsequently showed that the adsorption behaviour of natural sediments can be modelled via one or two surface reactions. Soon, the concepts of triple layer, diffuse layer and constant capacitance SCM were introduced to study metal adsorption on oxic sediment and soil (further references are given in Wen et al., 1998).

According to Wen et al. (1998), SCMs can be applied in two different ways to composite materials, like natural sediments. At first, sediments can be treated as an integrated whole with adsorption of metals described as complexation with average surface functional groups. The second way considers only main composites that are supposed to dominate adsorption interactions. Accordingly, the *'capacitance model'* assigns only one plane in the interface and all specific adsorbed ions are assigned into that surface plane. The electrical double layers are of a Helmholtz-type (detailed references are given), with three specific parameters, including an electric capacity (C), and two intrinsic acidity constants (pK_{a1}^{int} and pK_{a2}^{int}). The *diffuse layer model* assigns two planes in the interface: a surface plane for adsorption of H^+-ions, OH^- and all specifically adsorbed species (including metal ions), and a diffuse layer plane described by the Poisson-Boltzmann equation (see ref.). On the other hand, there are three planes in the interfacial region of the *triple layer model*: again a surface plane for adsorption of H^+, OH^- and strongly adsorbed ions, a near-surface plane for weakly adsorbed ions, and a diffuse layer plane representing the closest distance of approach of dissociated charges.

Wen et al. finally conclude that the model calculation as well as own experimental adsorption data so far indicate that *metal adsorption*, e. g. on river sediments, is *strongly pH-dependent.* A large percentage of adsorption occurs within a *narrow pH range*, the classical so-called “adsorption edge”. By increasing total dissolved metal concentration, the pH adsorption edge is consequently shifting to higher pH values, indicating a decrease of the adsorption affinity (see also section 5.5.4).

5.4.8 Speciation-bioavailability interactions in sediment-ingesting biota

During recent years, two different approaches have evolved to describe metal speciation and bioavailability interdependencies, i.e. absorption (or assimilation) efficiency (AE) and gut juice extraction.

We know today that bio-uptake of metals can occur both from dissolved and particulate sources, whereby the latter is a function of the assimilation efficiency (AE), of the metal concentration in ingested particles, and of the feeding rate. There seems to be no simple relationship between the binding strength of metals in sediment particles, and the AE.In oxidized sediments, TOC (total organic carbon) is known to reduce metal bioavailability. However, recent research shows that the influence of TOC on metal bioavailability is far from being understood and may strongly vary among metals and organism species. This may be partly due to the fact that sediment TOC studies mostly do not differentiate between humic, algal or bacterial matter, each of which can affect bio-uptake and accumulation of metals in a different way. For instance, humic matter decreases metal bioavailability due to high binding coefficients and high ligand concentrations, while the living organic sediment microflora may in contrast increase bioavailability.

To what extent and in what way gut physiology (e. g. pH, enzymatic activity, surfactant concentration, particle processing time and intensity, abrasional mixing) determines the AE is almost unknown and needs further research. Recent studies point to a strong influence of biological factors, like particle selection, flexible digestion, gut redox status, reproductive state, or gut fluid chemistry, on final metal bioavailability. There is also evidence that the ‘age’ of metal-sediment associations, beside gut residence times and readsorption of initially desorbed metals in the animal gut, may control AE, and/or minimize metal bioaccumulation. Also antagonistic effects between certain trace metals (as shown for Cd and Zn), or adaptive physiological *regulation mechanisms by the organisms themselves for essential metals, may affect AEs in aquatic biota.*

New experimental results from gut juice extraction studies confirm that bioaccumulation of metals may not be predictable solely based on their bioavailaibility. Experiments with digestive fluids obtained from deposit and suspension feeders, in combination with chemical extraction, showed that metal bioavailability varies among organism species according to the type of digestive physiology. These studies also elucidate the role of some major metal-binding sediment phases, like sulphides or Fe/Mn oxides, in that they can limit metal solubilization by the digestive fluid of deposit feeders.

Metal speciation and bioavailability studies are traditionally decoupled from toxicity studies due to the problem to relate tissue body burden directly to toxicity. In the same way it is difficult to relate bioavailability to bioaccumulation. During recent years, two different approaches have evolved to study metal speciation and bioavailability interdependencies in organisms ingesting sediments, i. e. the *absorption or assimilation efficiency (AE)* and *gut juice extraction*. In general, equilibrium partitioning models assume that sediments are not a direct but indirect source for metal accumulation in biota, and that they may act as a buffer or source/sink for metals in the water column and porewater. In this context, we also know that the relative importance of sediment vs aqueous uptake is highly specific for different groups of organisms.

5.4.8.1 Absorption efficiency (AE)

It is well accepted that *biouptake* can occur both from the dissolved phase, via respiratory membranes, and from particulates, via the gastro-intestinal tract, whereby the latter being a function of the *assimilation efficiency* (AE), i. e. of the metal concentration of ingested particles and of the feeding rate. According to Griscom et al. (2000), AE-values reflect per definition the bioavailability of a specific metal for a given animal under specific conditions (compare to section 5.1.2). It may offer a tool to integrate and quantify geochemical, sediment and species influences on the uptake of metals from food. AE's can be experimentally determined by *"pulse-feeding radioactive metal-labeled particles"* to test animals and by measuring the metal fraction retained after gut depuration. Griscom and co-workers (2000) showed that it can strongly affect the metal accumulation in mussels. They evaluated AEs of metals (Ag, Cd, Co, **Cr**, Se, and **Zn**) ingested by mussels from various sediments and sediment components, and the influence of geochemical properties which may affect metal bioavailability. In addition, the relationship between dietary metal assimlation and AVS binding was addressed in both anoxic and oxic sediments.

In oxic sediments where AVS does not control metal accumulation in porewater, it is supposed that *total organic carbon* (TOC) is reducing bioavailability of metals, in contrast to a report (ref. given by Griscom et al. 2000) indicating that bioavailability increases with the living organic sediment microflora. In their study, Griscom et al. observed that the influence of TOC on metal bioavailability was not consistent and varied among metals and test species. In particular, the experiments showed that sediment TOC did influence Cd-AEs, but not AEs for Zn and Cr. This may be partly due to the fact that sediment TOC studies generally do not differentiate between *humic, algal or bacterial matter*, each of which can affect AEs in a particular way. In this context we know that humic matter *decreases* metal bioavailability due to high binding coefficients and high ligand concentrations. In their study, Griscom et al. (2000) found AEs from bacteria-coated glass beads being slightly higher than from humic-acid coated beads. In this context, Robertson and Leckie (1999) also argue that it may be too difficult to predict the effect of humic substances on metal behaviour and the impact of ambient solution conditions on these effects at the same time, due to the *complex heterogeneous nature* (molecular weight, composition, structure) of the humic matrix.

We know that there is no simple relationship between the binding strength of metals for sediment particles and the AE. The work of Griscom et al. showed that sediment-bound trace metals were assimilated to varying degrees by suspension-feeding animals, ranging from 1 % (**Cr**) to 41 % (**Zn**). AEs of all trace metals associated with anoxic sediment were 1.5 times higher for the bivalve *Mytilus edulis* when compared to metals bound to oxic sediment (except for Ag). It was also observed that the exposure time of metals in the sediment correlated with the decrease in AE by *M. edulis* for Co, Cd and **Zn** (except for Ag). Results from sequential extraction showed at the same time that the labile fraction of all metals decreased over time (*"aging"*; see further below), which coincided with a decrease of the AE in the bivalve, due to the observed lower bioavailability. Also interesting that 1.5 times more Cd than **Zn** was extractable in the labile fraction (water rinse, exchangeable and weak-acid extractable metals), although AE of **Zn** was twice that of Cd. Somewhat surprising was that sediment organic matter apparently did not show a consistent influence on the AE among the selected metals, probably due to the fact that TOC was not further characterized. No statistically significant relationship was observed between the mean AE (among particle types) and the mean desorption of metals, as obtained from desorption experiments (with seawater adjusted to pH 5 and 8).

It is accepted that *geochemical influences* affect porewater concentrations, but these influences on dietary uptake are suppposed to be less extreme. The authors argue that correction factors appropriate among species and metals (like AVS or TOC normalization) are not applicable for dietary uptake. They also point out that the observed AE of metals in bivalves is less or equal to AE-values reported for periphyton or seston (see ref. given therein). Also that metals were less assimilable from anoxic than from oxic sediments in *M. balthics*, but still bioavailable, in contrast to what was the case for *M. edulis*. Higher AEs for Cd and Co in *M. edulis* from anoxic than from oxic sediments were unexpected given the supposed strong binding by sulphide and their slow reoxidation (days). The authors explain that *M. edulis* has a stronger reducing gut than *M. balthica* as indicated by preliminary experiments. In how far and what way the *gut physiology* (e. g. pH, enzymatic activity, surfactant concentration, particle processing time and intensity, abrasional mixing) may contribute to the observed AE differences is almost unknown and needs further research. Indeed, several studies (also cited here) point out to a strong effect of *biological factors*, like particle selection, flexible digestion, gut redox status, reproductive state, or gut fluid chemistry on the final metal bioavailability (see following section).

In this context, Griscom et al. addressed also the *'age'* of the metal-sediment association and could show that it had a pronounced effect on the AE in *M. edulis* (see section 5.5.6). Similar effects appeared after 35 days also on *M. balthica*, suggesting additionally species-specific differences. For example, we know that *M. balthica* has a *longer gut residence time*, which may lead to stronger absorption, which in turn may diminish or *mask the effect of exposure time*. But also *readsorption in the gut* of the initially desorbed metal ions, e. g. in the presence of surfactants, may *minimize assimilation* in animals. On the other hand, desorption experiments showed that the gut juice of different animal species varies in its ability to extract sediment-bound metals (see also following section). A progressive redistribution of added metals over time into more resistant phases in the sediment was observed by SEP, emphasizing the importance of sediment aging on metal AE. It is speculated that trace metals may even become lodged over time into *sedimentary mesopores*, a mechanism proposed by Mayer (1994) (cited by Griscom et al. 2000) for the observed loss of labile organic matter in sediments.

In a recent survey on extremely contaminated sediments from a marine coastal bay, also Fan et al. (2002) tried to examine the control of sediment geochemistry on the assimilation (expressed as AE) of Cd, **Zn** and **Cr** in marine mussels and clams by combining a sequential extraction method (the 'Tessier' procedure), with the 'radiotracer pulse-chase feeding technique', and the AVS approach. *Metal assimilation efficiencies* were measured in clams and mussels feeding on contaminated sediments radiolabeled with ^{109}Cd, ^{65}Zn and ^{51}Cr. A clear relationship between metal partitioning and total concentrations was only observed for Cd and less for Zn, in that the most labile phases increased with increasing total concentration. This confirms the empirical observation that metals released by human activities in general bind to more labile forms in the sediment. In contrast, but not surprising, **Cr** was almost completely (72-91%) bound to the residual phase and its sediment partitioning did not reveal a distinct dependence from total concentration. A significant correlation between the AE in both clams and mussels and the partitioning of metals into easily exchangeable and reducible phases was only established for Cd, inspite of a negative correlation between total sediment Cd and AE. Fan et al. suggest that this may be due to either *saturation of Cd ligand binding sites in the gut* or due to an *antagonistic interaction* between Cd and Zn occuring in very high concentrations in the sediment (up to 10.000 ppm!), in that Zn successfully competes with Cd for available gut binding sites. The decrease of Cd concentrations in deposit-feeding invertebrates with increasing Zn concentrations has been reported earlier and is a well known phenomenon (ref. given). The increasing Cd AE with increasing partitioning of this metal in the easily reducible phase may be due to increasing desorption of this metal in the bivalves gut. In contrast to previous studies with artificially prepared sediments, the authors did not find a significant relationship between the AEs observed for **Cr and Zn** and their partitioning in easily exchangeable and reducible phases. The authors interpret this discrepancy by the influence of other geochemical factors existing in natural sediments, like Fe, TOC or varying total metal concentrations, but also by the greater complexity of Cr speciation (with two redox species!), or by possible adaptive physiological regulation mechanisms for Zn (as an essential element!), which has been observed for many aquatic invertebrates.

In addition, differences in [SEM-AVS] did not significantly affect AEs of Cd, Zn and Cr. As shown by others, sediment-dwelling oligochaetes and insects take up most of their Cd from the overlying water column, which was also here the main source for metals, mainly due to the particular behaviour of these aquatic invertebrates, such as *burrowing and irrigation*. Lee et al. (2000a und 2000b, see section 5.4.3) (cited in Fan et al. 2002) recently found that AVS-bound metals were assimilated by clams with a similar efficiency as metals bound to oxic sediments, and that metal accumulation in the clam was not related to the SEM/AVS ratio but to metal extractability. The authors speculate about the reason for the observed lack of correlation between AE and AVS, and argue that the very high metal contamination would make it difficult to create [SEM-AVS] < 0. Consequently, speciation influences on metal bioavailability may be also confounded by the degree of contamination. However, due to differences in the partitioning of spiked and natural sediments (see above), extrapolating metal AEs by the use of radiotracer techniques to field conditions should be made with great care.

5.4.8.2 Gut juice extraction

Yan and Wang (2002) used a simple kinetic model and could show that *sediment ingestion* by the deposit-feeding peanut worm *Sipuncula nudus* was the dominant source for metal accumulation. More than 96% of Cd, > 89% of **Cr** and > 85% of **Zn** bioaccumulation could be predicted from sediment ingestion. However they found low uptake rate constants for *S. nudus* with regard to these metals. The uptake rate for Zn increased with increasing water concentrations. The corresponding AEs were 6-30% for Cd, 0.5-8% for Cr, and 5-15% for Zn.

The metal fraction assimilated by the worm was generally lower than the fraction extracted by the gut juice indicating that AEs may also depend on the actual amount of metals extracted in the animal gut from the ingested sediment. Metal desorption from radiolabelled sediment was measured by gut juice extraction obtained from *Sipuncula* and yielded up to 63% of Cd, but only up to 4% of Cr. A significant correlation between AE and gut juice extraction was only observed for Cd, but not for extractable Zn and Cr, indicating at least for the latter metals an *uncoupling between desorption and assimilitation in the gut* and confirming that the bioaccumulation of metals may not be predictable solely based on their bioavailaibility. Zn AEs from most investigated sediment types remained within a narrow range (6-9 %) despite obvious variations of Zn extraction in the gut juice. The extremely low fraction of Cr extracted by the gut juice suggests a strong association of this metal (which indeed often exists in residual phases) with the ingested sediment. However, one additional explanation for the observed differences between AE and gut juice extractable metals may be that the gut juice was collected by centrifugation, which may have altered the original enzyme activity.

More recently, Fan and Wang (2003) compared different extractants (normal and acidic seawater pH5, seawater with 1 % sodium dodecyl sulfate, and gut digestive fluid from *Sipunculus nudus*) with operationally defined geochemical phases (exchangeable, reducible and carbonate phases) in contaminated coastal sediments. Extracting Cd, Cr, and Zn by gut juice did not correlate with the concentration of SEM or SEM-AVS, indicating that significant differences may exist between the chemical (AVS) and biological (gut) availability of metals.

To demonstrate that the bioavailability of sediment **Cu** is not only determined by the particular sediment geochemistry, but also by the *digestive physiology* of exposed organisms, Chen and Mayer (1999) used a *biomimetic* approach which involved Cu extraction with digestive fluids obtained from 2 deposit feeders and 1 suspension feeder, in comparison to AVS-extraction, to finally define Cu bioavailability as the amount of Cu solubilized by the gut juices. One major goal was to test if sulphide plays a role in limiting Cu solubilization by the digestive fluid of the used deposit feeders considering that sulphide may act as a strong competing Cu-binding phase and hence prevent Cu toxicity in estuarine sediments with varying SEM_{Cu}-AVS values (no information was given if these sediments were oxic or anoxic). The experimental results showed that Cu bioavailability varied among organism species having unequal types of digestive physiology, but increased significantly at $SEM_{Cu} - AVS \geq 0$ according to the AVS premise. As an example, the amount of copper bioavailable to the digestive fluid of *Arenicola marina* (deposit feeder) was higher in sediments with SEM_{CU}-AVS > 0. A SEM_{CU}-AVS range of 0.4 and 1.5 μmol/g (sed) was necessary for the 'holothuroid' gut fluids (of *Parastichopus californicus* and *Cucumaria frondosa*) before a significant increase in Cu solubilization was observed suggesting AVS is limiting Cu bioavailability.

Positive SEM_{CU}-AVS values may also point out that other additional binding phases than AVS, like organic matter, may successfully compete with *dissolved gut ligands*, or that Cu(I) and Cu(II) sulphides are present in the sediment (with a smaller amount of sulphide present in Cu_2S, in contrast to CuS) (see also Simpson et al., 2000b). The amount of Cu available for the digestive fluids was less than that measured as SEM_{CU}-AVS, suggesting that only a fraction of the HCl-soluble non-AVS bound Cu was solubilized during the digestive process (see Table 5.11), and again the more conservative nature of the AVS concept (cf. section 5.4.3.3). This fraction decreased in the order *Arenicola* > *Parastichopus* > *Cucumaria*, which is consistent with the decreasing concentration of the strongest *Cu binding ligands* (i. e. *histidine*) found among the digestive fluids of the tested organisms. The authors conclude that sedimentary Cu availability is not only a measure of the applied geochemical factors (e. g. AVS), but varies also according to the digestive physiology of exposed organisms.

Table 5.11. Example of Cu released by 1 N HCl, gut fluids inArenicola marina, Parastichopus californicus, Cucumaria frondosa and sea water from harbour (BBH and PLH) and estuarine sediments (BIW), in comparison to total sedimentary Cu and acid-volatile sulphide[a] (AVS) (from Chen and Mayer, 1999)

Sample	1 N HCl	A. marina	*P.californicus*	*C. fron-dosa*	seawater	total Cu	AVS
BBHI (I,sand)	7.01±0.30	1.65±0.43	0.78±0.37	0.19±0.05	0.006±0.001	8.59±0	0.48±0.08
BBH2 (I) (coarse sand)	1.19±0.02	0.18±0.01	0.16±0.01	0.04±0.00	0.001±0.000	1.20±0.01	0.02±0.01
BBH3 (I,sand)	3.66±0.09	1.34±0.24	0.22±0.13	0.14±0.03	0.006±0.002	6.00±0.14	0.84±0.21
BBH4 (I,sandy silt)	5.50±0.09	1.74±0.64	0.59±0.07	0.07±0.01	0.020±0.002	9.56±0.34	0.19±0.02
BBH5 (I,silt)	0.70±0.00	0.09±0.02	0.00±0.00	0.00±0.00	0.000±0.000	1.79±0.02	0.34±0.05
BBH6 (I,clay)	1.57±0.06	0.44±0.04	0.01±0.01	0.00±0.00	0.001±0.000	3.31±0.16	0.12±0.02
BBH7 (I,coarse sand)	0.03±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.000±0.000	0.06±0.00	0.39±0.12
BBH8 (S,silty clay)	0.28±0.01	0.07±0.01	0.02±0.01	0.00±0.00	0.000±0.000	0.28±0.00	0.16±0.04
PLH-F (I,silt)	0.40±0.01	0.05±0.00	0.00±0.00	0.00±0.00	0.000±0.000	0.48±0.01	2.24±0.39
PLH-B (S,silt)	0.18±0.01	0.03±0.00	0.00±0.00	0.00±0.00	0.000±0.000	0.18±0.00	1.27±0.08
BIW1 (S,silt)	0.15±0.00	0.02±0.00	0.00±0.00	0.00±0.00	0.000±0.000	0.50±0.01	0.09±0.01
BIW2 (S,silt)	0.14±0.01	0.01±0.00	0.00±0.00	0.00±0.00	0.000±0.000	0.30±0.01	0.07±0.00
BIW3 (S,silt)	0.10±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.000±0.000	0.30±0.01	0.09±0.03

[a] All data as mean ± 1 S.D. (n = 2) with a unit of μmol/g sediment. I = interidal, S = subtidal

Incubation of digestive fluids with Cu-binding model phases (geothite and sulphide) confirmed the relative *unavailability of sulphide-bound Cu*, which means a reduced Cu exposure to subsurface deposit feeders feeding on anoxic sediment in comparison to surface feeders. Less Cu was released from sulphide than from geothite by the digestive fluids demonstrating sulphide as the stronger Cu-binding phase. This is in good agreement with the size of the corresponding Cu-binding constants of the sulphide ($\log K_{CuS}=36.1$) and hydroxyl group ($\log K_{CuOH}=6.5$). It is known that field sediments may contain a mixture of α-FeOOH and sulphides over a wide range of concentrations reflecting varing sedimentary redox conditions, which in addition to organism species may influence the bioavailability of Cu in sediments for a given total concentration (see following section).

Chen and Mayer (1999) state that neither the *deposit feeding biology* nor the digestive physiology have been sufficiently examined yet by current research approaches designed to assess metal bioavailability, although we know that digestive fluids enhance metal solubilization, and vary among different deposit feeders. In addition, *histidine containing proteins and peptids* in digestive fluids have been found particularly responsible for mobilising and complex-binding sedimentary Cu. But relatively little is known about their role in controlling metal bioavailabilities, bioaccessibility and uptake in natural biota. Moreover, to what extent released metals are influenced by the ratio of *gut ligand concentration* and actual sediment metal load, or by the reaction time between sediments and digestive fluids, remains still unknown. The fact that 1 N HCl (SEM) released much more Cu than the digestive fluid of the 3 tested deposit feeders, may be also partly due to the acidic extraction pH causing a harsher attack than the neutral pH in the gut. Also the amount of Pb, Zn, Cd and Ni released by digestive fluids was insignificant compared to Cu, although Pb and Zn reached a significant fraction in the 1 N HCl fraction.

5.4.9 Redox effects

The geochemistry of trace metals in anoxic sediments is different from the geochemistry of metals in oxidized sediments. In particular, the upper 3 cm of aquatic sediments represent a highly reactive zone of biogeochemical transformation. Sediment red-ox potential (expressed as E_h *value) can be considered as a bulk electrochemical measure for oxidized and reduced metal-binding forms occurring in the sediment pore-water, which may integrate all relevant metabolic and transport processes and effects in the sediment. Red-ox characteristics may influence behavioural processes of sediment dwelling organisms and, thus, metal bioavailability. Moreover, these metabolic and behavioural processes advect particles and water between bottom water and the deep sediment, and finally define the overall structure of the pore-water chemistry. In this way, a full spectrum of biogeochemical conditions of solubility, reactivity and microbial metabolism of organic matter and compounds is created, which finally defines metal solubilities, and steadily pushes the* E_h *horizon in the sediment profiles towards more oxidizing conditions.*

The geochemistry of trace metals in anoxic sediments obviously is different from the geochemistry of metals in oxic sediments, with corresponding implications for metal speciation and bioavailability. According to Griscom et al. (2000) metal partitioning and bioavailability seems most complex in oxic sediments. As demonstrated before, anoxic sediment conditions with metal sulphide formation alone do not appear to eliminate metal uptake from the diet by benthic invertebrates (see above).

Davis et al. (1998) considered the *sediment redox potential* (expressed as E_h value) as a bulk electrochemical measure of oxidized and reduced metal-binding compounds existing in sediment porewater, which integrates all consequences of *metabolic and transport processes* occuring within benthic communities. The authors discuss and speculate about the importance of rather simple but often overlooked behavioural processes of sediment dwelling organisms, which may influence redox characteristics and so metal bioavailability (compare section 5.4.6). For example, *motile benthic macrofauna* ingest and transport particles, *ventilate* deep burrows in anoxic sediments with the overlying oxic water, while *sedentary suspension-feeding organisms* deposit suspended organic matter onto the sediment surface. All these metabolic and behavioural processes *advect particles and water* between bottom water and the deep sediment and finally define the overall structure of porewater chemistry. In this way these processes create a full spectrum of biogeochemical conditions of solubility, reactivity and microbial metabolism mineralizing organic matter and compounds, which finally define metal solubility, and push vertical E_h profiles steadily towards more oxidizing conditions. The authors stress that the use of *standard E_h probes* inserted downward through the bioturbation zone of a sediment can provide a general measure of porewater chemistry appropriate for screening purposes.

In the following, some recent examples are given to underline once again the importance of the prevailing redox conditions for the speciation of trace metals in aquatic sediments.

Donahoe and Liu (1998) found a good spatial corrrelation between trace metals and Fe and Mn in wetland porewater and sediments suggesting that adsorption and desorption reactions from Fe and Mn oxyhydroxides are controlling dissolved metal distribution. However, equilibrium adsorption modelling alone did not explain the observed distribution. On the other hand, *redox kinetic modelling* was suggesting that relative rates of ferrous Fe oxidation and reductive dissolution of ferric iron in sediment may coexist and so determine the final porewater distribution of metals. Also Xue et al. (1997) could confirm the relevance of redox-controlled processes at the sediment-water interface for the cycling of **Cu** and **Zn** in eutrophic lakes. *Hypolimnetic oxygenation* enhanced the release of sediment-Cu (from sulphide oxidation), but at the same time accelerated entrapment and deposition of Cu and Zn by freshly formed Mn and Fe oxides. Aström (1998) studied the behaviour of trace metals in sulphide-bearing fine-grained sediments exposed to atmospheric oxygen. On sediment oxidation high portions of Co, Mn and **Ni**, intermediate of **Cu** and low portions of Fe, Al, **Cr** and V were released from the sediment. The extent of release seemed to depend on the drop in pH (as a consequence of sulphide oxidation), the actual amount of metals associated with (H_2O_2-extractable) metal sulphides, and on the sum of the amount of metals associated with reduced sediment phases and those adsorbed on soil compounds. The author stressed that any artificial drainage of these sulphide-bearing wetland sediments may cause extensive leaching in particular of Co, Mn and Ni.

That the upper 3 cm of sediments taken from a mining lake represent indeed a *highly reactive zone* of biogeochemical transformation processes (for example, expressed by enhanced concentrations of lipid phosphate, degradable organic matter, DOC and Fe^{2+} in the porewater and a drastic change in sulfur isotope ratios) was likewise documented by Friese et al. (1998). The occurrence of Fe and SO_4-reducing processes resulted in a distinct pH increase with sediment depth.

In another study, Gianmarco et al. (1997) assessed the seasonal variation of the redox potential represented by the mobility of sulphide in a dystrophic lagoon sediment. In general, they observed high reactive Fe (II, III) concentrations and low SO_4-reduction rates in the sediment porewater. Particularly in winter and spring, reactive Fe^{3+}-levels were high, whereas in summer free sulphides prevailed in porewater although most of the reactive Fe^{2+} was not sulphide-bound, indicating that the reactive Fe pool is not as a whole available to buffer against sulphide release. For this reason, the authors judge the reactive Fe pool not as a good measure of the true *redox buffering capacity* in sediments.

Lee et al. (1997) used distribution coefficients (k_d) to assess the *relative mobilities* of metals in retention pond sediment along a motorway. They found a vertical decrease of k_d for **Zn** (and Cd), and an increase for Pb. Based on established k_d-values the investigated sediments showed different relative mobilities in the upper (Mn>Zn>Cd>Pb) and lower sediment layer (Mn>Cd>Zn>Pb). If and to what extent the observed vertical distribution of metal mobilities (expressed as k_d value) in the sediment follow prevailing redox and pH gradients was unfortunately not demonstrated by the authors.

5.5 In soils

5.5.1 Introduction

Like in sediments, metals in soils are part of a complex mixture of solid phase compounds of varying particle size and morphology, including discrete mineral phases, coprecipitated and sorbed species associated with soil minerals and organic matter, and dissolved species complexed by a variety of inorganic and organic ligands, including the vast variety of soil macro- and micro-biota. The distribution of metals among these phases and components, and their physical relation to the soil, control dissolution properties of the metals and hence their bioavailability.

One of the main *differences between soils and aquatic sediments* is that the latter are in intimate contact to an overlying water body, while soils are directly exposed to the atmosphere. As a consequence, soils are the result of *natural weathering processes*, while sediments are build up by solutes and particulates entering or formed in the water column. Although geochemically rather similar, this difference makes that soils and sediments are subjected to almost completely different physical, chemical and biological conditions, with the result that the properties of solid phases and the kinetics of reactions at the solid-liquid interface dominate soil solution chemistry. However, their underlying similar geochemistry and mineralogy was one reason, why methods developed and used for metal speciation are rather the same for both types of substrates, and why soil scientists working on metal speciation often are also involved in corresponding studies on sediments, or *vice versa*. Beside some more classical soil extraction procedures, which are almost exclusively used to estimate plant-uptake and bioavailability of certain trace elements (like the $CaCl_2$-, EDTA-, or DTPA-extraction), current speciation methods are almost equally used for both soils and sediments. In contrast, techniques to obtain soil or sediment porewater obviously differ from each other, as do laboratory-based bioassays developed to assess the ecological risk of metal contamination, due to obviously different habitat conditions (i. e. soil/air versus sediment/ water) existing in either systems. A enhancing cooperation between soil and sediment scientists in the field of trace metal speciation would surely contribute to improve our present understanding of the influence of processes and factors that control the intricate interaction between metal species, solid substrate and organisms, as well as of existing analytical and modelling approaches designed to assess and predict their potential impact, if environmental conditions change. In the following, we will focus on those soil parameters and speciation methods that have evolved as relevant during recent years to successfully characterize the bioavailability of metals.

5.5.2 Metal adsorption and partitioning

Like in sediments, metals in soils are part of a complex mixture of solid phase compounds of varying particle size and morphology, including discrete mineral phases, co-precipitated and sorbed species associated with *soil minerals and organic matter, dissolved species complexed by a variety of inorganic and organic ligands, including a vast variety of macro- and micro-organisms. Although geochemically similar, soils are exposed to the ambient air and the result of natural weathering processes, while sediments are exposed to water and built up by solutes and particulates entering or formed in the overlying water. Based on their geochemical similarity, similar speciation methods for soils and sediments have been developed.*

A first approximation of the speciation of metals in soils can be achieved by assessing their partitioning to the soil particulate matter, and to soil solution. Unlike organic contaminants, metal adsorption in soils is much metal- and soil-specific, meaning that the assessment requires the actual experimental determination of solid-liquid partitioning coefficients (k_d). Partitioning coefficients simply help to differentiate between metals dissolved in the soil solution and those bound to the soil particulate phase. From this, however, no information is available concerning the relative bioavailability of dissolved metal species. Recent studies emphasize that pH is still the most beneficial and universal site-specific k_d adjustment parameter, although the sorption capacity of a sorbent also depends on a variety of other soil parameters. This is why k_d values from low-contaminated soils cannot be applied to assess the partitioning of metals at contaminated sites. Measured k_d values depend in particular on total dissolved metal concentrations and on dissolved organic matter (DOM), why every factor influencing these main parameters also will affect metal partitioning and speciation. Today, competitive adsorption models rather than solid-solution ratios (as expressed by k_d) are increasingly used to predict dissolved metal concentrations.

The realization that metals contribute to plant uptake also from pools other than soil solution has been delayed during the 60ies, mainly due to the lack of simple realistic methods to assess the rate and extent to which solid-bound metals are released and taken up by biota. But we now know that the bioavailable pool of metals includes the *free metal ion* in solution, and a *labile pool*, which buffers the free metal concentration in solution, and includes both species on soil solids or soluble organic matter (DOC). A first approximation to the speciation of metals in soils may be simply done (like for sediments) by partitioning between metals bound to the soil particulate matter, and those dissolved in soil solution. It is the *dissolved metal pool* that is in general reflecting metals that are easily taken up by plant roots or other soil biota, or leached from the soil, to contaminate ground and surface waters. However, unlike organic contaminants, metal adsorption in soil is much metal- and soil-specific, and partitioning requires the actual experimental determination of solid-liquid partitioning coefficients (k_d). It should be noted, however, that such site-specific k_d's may not ensure a correct assessment of the fate of metals under changing conditions, but they allow an estimate of dissolved metals, and to predict metal mobility (compare also to section 5.2.2 and 5.4.7.2).

Sauve et al. (2000) provided a compilation of soil solid-liquid metal partitioning data to identify default k_d-values (L/kg) for various risk assessment models, but also to evaluate the *dependence of* k_d *on soil characteristics*, like pH, total metal load and organic matter. The authors point out that *sorption models* using a single-valued k_d approach presume that the sorption capacity of a sorbent is independent of any soil characteristics, which we know that it is not. An example is given (ref. therein), where **Zn** and Pb reacted in opposite ways to increasing total soil concentrations. In this case, higher dissolved Pb concentrations increased adsorbed Pb and the corresponding k_d, in contrast to Zn, where higher soil levels decreased k_d values reflecting a lowered affinity of solids for Zn at higher concentrations. Also for this reason, k_d values from low-contaminated soils cannot be applied to study the partitioning of metals at contaminated sites. In addition, it remains difficult to distinguish between the influence of pH and other soil characteristics, as they are mostly autocorrelated.

The authors (Sauve et al. 2000) further explain that k_d values above all depend on total dissolved metal concentrations, i. e. the sum of *free metal pools*, *inorganic ion pairs*, like $MeOH^+$, $Me(OH)_2^0$, $Me(OH)_3^-$, $MeHCO_3^+$, $MeCO_3^0$, $Me(CO_3)_2^{2-}$, $MeNO_3^+$, $MeCl^+$, $MeSO_4^0$, etc., and of *dissolved organic matter (DOM) complexes*, which in turn are particularly affected by pH. DOM strongly influences metal solubility and hence the level of total dissolved metals (the authors give an example, where > 98 % of dissolved **Cu** was bound to DOM in non-acidic soil solution). So, for example, every factor influencing soil organic matter (SOM) will also affect metal solubility. Another example is given, where Ca was found to promote the coagulation of DOM, which in turn reduces its solubility, and so also the solubility of other trace metals bound to DOM. In contrast, Ca and other cations compete with metals for surfaces and so may again enhance their solubility. The partitioning of SOM to particles, in particular its fulvic:humic ratio is strongly a function of the given soil solution pH. *Multiple binding site models* have been developed (ref. given) to adequately describe the interaction between organic matter and metals over a wide range of concentrations.

Also *inorganic ligands* are recognized for their capacity to solubilize metals depending on their concentration and corresponding dissociation constants. However, the influence of main geochemical parameters (i. e. of pH, DOM and competing or complexing ions) on metal adsorption is not well defined by the *k_d-approach.* k_d values simply help to differentiate between metals dissolved in the soil solution and those bound to the solid phase. From this, no information is available, for instance, on the relative bioavailability of various dissolved metal species. As part of their study, Sauve et al. (2000) applied a *'competitive adsorption model'* to predict the solubility of Cd, **Cu**, Pb and **Zn** in soil, assuming that free metal (Me^{2+}) and H^+ ions compete for sorption on available soil exchange sites. The study confirmed that most of the observed variability depends on solution pH and on total soil metal content, beside DOM. It is suggested that a long-term stabilization of the collected field soils will move metals closer to equilibrium regarding sorption, precipitation or specific retention ("aging effect"). Their work further demonstrate that the linear relationship between k_d and soil solution pH can explain more of the observed variability than does total metal burden (i. e. between 29-47 % for Cd, **Cu** and Pb, and between 56-58 % for **Ni** and **Zn**). These studies emphasize that pH is still the most beneficial and universal site-specific k_d adjustment parameter. The authors stress, however, that competitive adsorption models are actually predicting dissolved metal concentrations and not simply solid-solution ratios (as expressed by the k_d). For this reason it is supposed to use adsorption models rather than k_d values to directly predict dissolved metals, when assessing the environmental risk and fate of metals in contaminated soils.

There is an overwhelming evidence today that the *lability of metals* (i. e. their mobility and availability) vary greatly with the properties of a particular soil for similar total metal concentrations. In order to evaluate the effect of different soil:solution extraction ratios and the role of soil properties on metal desorption processes, Yin et al. (2002) conducted desorption experiments. Their data showed that *distribution coefficients* ($k_d = M_{soil}/M_{solution}$) increase with increasing soil:solution ratios, as well as with soil organic matter (SOM). There was in particular a strong affinity of **Cu** to operationally defined dissolved organic matter (DOM) compounds, which was reflected by a linear correlation between the k_d for Cu and the k_d for DOM. Furthermore, the soil-solution distribution of **Ni, Zn, Cu^{2+}** correlated closely to SOM but not to DOM. So, *normalization* of the k_d-values for Zn, Cu and Ni by SOM in the same soil improved the linear relationship of non-normalized k_d-values and soil-pH. The authors conclude that (organic matter) normalized regression equations are more convenient to predict Ni and Zn solubility and the free Cu^{2+} activity in the soil as a function of pH.

Aldrich et al. (2002) studied the input of Zn and Cu into agricultural soils because of their increasing use in feed additives (manure), fertilisers and fungicides. Cu and Zn contents in the respective test soils are summarized in Table 5.12. From there it can be seen that the humic soil in 'Baltenswil' exceeds the Swiss soil guideline for total Zn and Cu concentrations by a factor of 2. Dissolved metal concentrations remained below the guideline, but increased in the drainage water during rain events. Dissolved Cu was almost completely complexed by organic ligands (> 99.9%), whereas dissolved Zn in the drainage water was partly *electrochemically labile*, and partly *complexed by strong organic ligands*. The authors resume that these metals are hardly bioavailable in the soil drainage water due to the very low free metal ion concentration found.

Table 5.12. Total and soluble Cu and Zn contents (µg/g dw) in two Swiss soils treated with manure, a Zn-containing fungicide (Propineb with 22.6% of Zn) and Cu salt (50% Cu) (from Aldrich et al., 2002)

Sampling site	Cu total[a] µg/g	Cu soluble[b] µg/g	Zn total µg/g	Zn soluble µg/g
Lindau	48	0.05	82	0.2
Baltenswil	78	0.2	284	0.3
Swiss guideline	40	0.7	150	0.5

[a] Extraction with 2 M HNO_3. [b] Extraction with 0.1 M $NaNO_3$

5.5.3 Chemical extraction and plant uptake

Chemical extraction procedures (e.g. sequential extraction schemes) are widely used for preliminary classification of soils and/or different soil strata with regard to the mobility and plant-availability of trace metals. However, the reliability of these procedures is often questioned, mainly due to readsorption of extracted metals and non-selectivity of methods.

Depletion of metals in the vicinity of plant roots promotes their transfer from solid phases into solution. Plants can actively mobilize essential metals, like Fe, Cu or Zn, from solid phases under deficiency conditions. Under non-deficiency or excess conditions, soil properties rather than plant physiology determine whether or not soil-associated metals will respond to local depletion. For this reason, an understanding of both *solution chemistry (i.e. free metal ion activity) and solid phase supply processes (e.g. as characterized by operationally defined phases) is necessary to be able to predict metal bioavailability to plants.*

A new approach, the effective concentration concept (C_E), tries to combine both aspects by means of the so-called 'diffusive gradients in thin films' (DGT) technique. A metal chelating resin is placed in the soil, to lower the local dissolved metal concentration, and hence to mimic plant uptake. Principally, this technique reduces, like a plant root, the local metal concentration in the soil solution and, hence, induces a metal supply from solid phases. So, fluxes to the resin and corresponding concentrations can be measured based more on kinetic than thermodynamic equilibria. Like plants, DGT responds to the labile metal pool resupplied from the soil solution and solid phases. Measured DGT fluxes can be quantitatively related to the effective metal concentration (C_E), a measure directly related to plant metal uptake, encompassing both soil solution concentration and its enhancement from the solid phase. Altough C_E is largely determined by soil properties and depends on deployment time and thickness of the diffusive gel layer, it may provide a measure of the potential hazard of metal-contaminated soils. Recent studies show that DGT-labile species exclude strongly bound organically complexed metals as well as colloidal species not supposed to be utilized by plants, but include labile solution species, like inorganic and fulvic complexes, and clearly reflect the supply of kinetically labile metals from solid phases.

So far, most soil scientists agree that the free metal ion activity of the soil pore-water, rather than dissolved or total concentrations of metals, controls metal bioavailability to micro-organisms and plants. However, new studies indicate that metal toxicity also depends on labile metal forms, and that information is still scarce about the factors and processes determining the intricate, dynamic relationship between free, labile and non-labile metal species. Due to the large size of the labile relative to the free metal ion pool, and the even larger metal reserve in soluble non-labile forms and various soil particulates, the free metal ion activitiy seems to be well buffered.

Chemical extraction procedures still provide a valuable *preliminary classification tool* to estimate mobilities and bioavailabilities of metals in soils, which is however based on rather arbitrary responses to distinct chemical reagents, and hence does not reflect true metal labilities. Like for sediments, these procedures are widely used to obtain a first estimate of the complex relationship existing between metals and the various soil compounds, in relation to the potential uptake of toxic metals by plants

(Kabala and Szerszen, 2002). Assessing the pool of solid-phase metal that can buffer dissolved metal concentrations was one reason, why researchers have sought to identify soil extraction techniques that measure that particular pool. However, no clear evidence was found yet to indicate which solid-phase may best correlate with uptake by plants or soil animals. At best, soil extraction methods can integrate an *indication of the soil capacity to supply a certain metal* (metal release rate) (Allen, 2002).

Qian et al. (1996) evaluated the bioavailability of metals (**Ni**, Co, **Cu**, Pb) in soils, from various rural regions in China, to plants (winter wheat *Triticum aestivum*, alfalfa *Medicago sativa*) in pot-culture experiments, under greenhouse conditions (after 40 days), by means of the *Tessier-method* (see section 5.4.2.1) and multiple regression. Total soil concentrations were as expected poorly correlated with plant metal contents. Also no significant correlation (except for Co) was observed between plant metal concentrations and individual soil fractions. However, a *stepwise multiple regression analysis* using combinations of various soil fractions as independent variables could predict the observed variability in plant uptake, indicating that SEPs (sequential extraction procedures), though operationally defined, may provide valuable bioavailability data for metals in soil. The corresponding regression equations for Ni, Cu and Pb are listed in Table 5.13.

In a recent review, Schramel et al. (2000) examined several single and sequential extraction schemes developed to assess **Cu** mobility and bioavailability in contaminated agricultural soils. They found that both EDTA and acetic acid are widely used as extractants for *plant-available Cu species*, while deionized water, NH_4NO_3 and $CaCl_2$ extract more electrostatically weakly bound metals representing mobile phases in the soil. By means of own experiments, no vertical Cu translocation was found in hopfields, although Cu was highly plant-available. 1 M NH_4NO_3 is now used as the *standard method* of assessing metal bioavailability for regulatory purposes in the Federal State of Baden-Württemberg, Germany (DIN 1995).

Concerning the determination of total metal concentrations in arable and non-arable soils, Hseu et al. (2002) very recently reviewed currently used *acid digestion methods*. They found that beside the classical ‘aqua regia’, different combinations of concentrated acids ($HClO_4$, HNO_3, H_2SO_4, HF) are used by most research groups to extract the total metal pool in soils (see for more details section 5.4.4).

Table 5.13. Multiple and single regression equations between plant concentrations (alfalfa, wheat) of Co, Cu, Pb and Ni and metal concentrations in particular soil fractions (from Qian et al., 1996)

Metals	**Regression equation**	r^2
Co	$Y_a = 0.413 + 10.3F_1{}^a$	0.776**
	$Y_{sw} = 0.014 + 1.99F_1$	0.998**
	$Y_{rw} = 1.53 + 28.2F_1$	0.626**
Ni	$Y_a = -0.345 + 11.4F_1 + 0.124F_{2+3+4}$	0.778**
	$Y_{sw} = -0.114 + 2.46F_1 + 0.0268F_{2+3+4}$	0.790**
	$Y_{rw} = -0.269 + 12.7F_1 + 0.114F_{2+3+4}$	0.964**
Cu	$Y_a = 0.939 + 0.368F_1 + 0.0794F_{2+3} + 0.0655F_4$	0.791**
	$Y_{sw} = 1.054 - 2.95F_1 + 0.131F_{2+3} + 0.126F_4$	0.834**
	$Y_{rw} = 1.10 – 3.07F_1 + 0.138F_{2+3} + 0.119F_4$	0.822**
Pb	$Y_a = -5.88 +0.181F_{3+4}$	0.472*
	$Y_{sw} = -0.816 + 0.0278F_{3+4}$	0.524*
	$Y_{rw} = -12.1 + 0.460F_{3+}$	0.411*

** and * denote significance at the 5 and 10 percent level, respectively. [a]Extraction according Tessier et al. (1979): F_1 = $Mg(NO_3)_2$-extractable, F_2 = CH_3COONa-extractable, F_3 = NH_2OH HCl-extractable, F_4 = H_2O_2/HNO_3-extractable, F_5 = residual

The reliability of sequential extraction procedures has been questioned also for soils mainly due to the obvious *redistribution* of the extracted metals during the procedure. Although there have been many attempts to *standardize* a soil-extraction protocol, the ideal extraction reagent will always depend on the specific needs of each study (see recent review by Reuther 1999, and Allen 2002, and compare to section 5.4.4). Van Herck et al. (2000) used the 'MINTEQA2' chemical speciation code to predict metal concentrations in leaching soil solutions, minerals that precipitate and the pH of the leaching solution at equilibrium. By comparing the calculated and experimentally obtained leaching data with results derived from a sequential extraction scheme, the authors could verify the accuracy of the extraction method they used. Ho and Evans (2000) used radiotracer techniques to quantify the observed redistribution of **Zn** and Cd during a modified 'BCR' extraction procedure ((BCR = EU Bureau of Chemical Reference Materials; ref. given therein). In the soils examined, about 20-30 % of Cd and Zn released from the acid-soluble fraction of the soil was readsorbed to the following reducible oxide phase. However, most readsorption for Zn and Cd occurred already after the 1st extraction step and the exact extent of readsorption varied with soil type. The authors infer, however, that the extent of readsorption was less than previously announced and does not invalidate SEPs for these metals. The 'BCR protocol' was used because it is one of the more standardized extraction schemes today and so easier to perform and to reproduce. In addition, a certified reference sediment (CRM 601), available from the 'EC Measurement and Testing Programme' was used as standard against the 'BCR procedure'.

By adding *radioisotope tracers* of Zn and Cd into the extraction reagents, the authors could directly quantify redistribution effects and consequently correct the results for these metals. But radiotracer metals are not introduced in the same form as that in which their stable counterparts may occur naturally and hence do not represent the true actual metal partitioning. They can just be an indicator of the metal in solution, but not in the soil. Isotopic exchange occurs very rapidly and a steady-state partitioning of radiotracers (Zn and Cd) among the various soil fractions is obtained within minutes. For this reason, the authors conclude that, although partitioning of added radiotracers can not be used as an accurate substitute for stable metal isotope measurements, it is possible to make soil-specific corrections with regard to readsorption effects and to recompute metal partitioning in the BCR soil. By this way it seems possible to yield true amounts of operationally defined soil fractions.

Conder and Lanno (2000) used a weak electrolyte (0.1 M $Ca(NO_3)$) soil extraction method and could demonstrate that extractable metals (Cd, Pb and **Zn**) in spiked artificial soil were precisely related to the LC_{50} toxicity to the earthworm *Eisenia fetida*. The authors postulate that this type of soil extraction represents a good surrogate measure of the metal bioavailability in spiked soils. In addition, *mixture toxicity* measured by the 'toxic unit approach' (TU) could well reflect the *additivity* of single observed toxic effects (see also section 7.2).

Wang et al. (2002) used a sequential chemical extraction scheme to demonstrate possible differences in heavy metal speciation and bioavailability (**Cr, Ni, Zn, Cu**, Pb and Cd) occuring between the *rhizosphere and bulk soils*, as well as between air-dried and wet soils. Speciation differences were observed for all elements, both between the rhizosphere and bulk soils, and between air-dried and wet soil samples. Correlation analysis indicated a better correlation between the exchangeable/carbonate-bound metal fraction in the rhizosphere wet soil to the plant metal concentration, in comparison to air-dried or nonrhizosphere soils. Therefore, the authors recommend to apply *rhizosphere wet soils* in future metal speciation and bioavailability studies on soils. Interesting in this context also another finding of the authors that precipitation of $Fe(OH)_3$ due to the liming of metal-contaminated forest soils prevented an increase in soil pH, and was the reason why only minor effects on the free and exchangeable Cu and Ni pool ($BaCl_2$ + EDTA-extractable) were observed.

Urban soils often exceed trace metal regulatory levels. But the actual threat posed by metals depend on their speciation in soil solution rather than on total concentration. Murray and Hendershot (2000) found up to 59 % of the dissolved Cd in *urban soil solution* in a free ionic form. In contrast, most of **Cu**, Pb and **Zn** occurred as organic complexes, and >40 % of **Ni** as inorganic complexes at pH values > 8.1. A multiple regression analysis revealed that neither the free nor the dissolved or total soil metal pool was a good predictor for the metal uptake by plants in the field. However, it was shown that pH and total metals in soil were significantly correlated with the activities of *free metal ions*, except for Cd^{2+}, which only had a weak correlation with soil pH. Chemical extraction and analysis indicated that the selected trace metals were mainly in stable solid forms and bioavailability was extremely low in these urban soils.

There are many processes affecting the *supply of metals to plants*, including diffusional and consecutive transport to roots, which encounter fresh surfaces on their way through the soil, but also including the influence of particular *root microenvironments and exudates*. Depletion of a metal concentration in the vicinity of roots allows the *transfer* from solid phases into solution. And kinetically labile solid phase metals allow a corresponding rapid transfer into solution. Under deficiency conditions, plants can actively *mobilize* essential metals, like Fe, **Cu or Zn** from such solid phases. Under nondeficiency or excess conditions soil properties rather than the plant determine if a metal fraction responds to local depletion.

For this reason, to predict metal bioavailability to plants, an understanding of both *solution chemistry* (i. e. the free ion activity) and *solid phase supply processes* (e. g. by operationally defined phases) is required. However, applying the FIAM to soil systems is not as simple as its application to aquatic systems, due to the lack of considering dynamic processes responsible for maintaining that free metal activity, including biological uptake. Metal bioavailability in soil solution seems not only to depend on the chemical availability but also on the soil's capacity to supply and maintain a certain level of free ionic metals. Zhang et al. (2001) tried to combine both aspects by introducing the *effective concentration* (C_E) concept, which includes the use of the 'diffusive gradients in thin films' (DGT) technique. Placing a metal chelating resin into the soil, lowers the local dissolved metal concentration and hence *mimics plant uptake*. Principally, the same is happening when using the DGT technique, which locally reduces, like a plant root, the metal concentration in the soil solution and, hence, *induces a metal supply from solid phases* (see Figure 5.2).

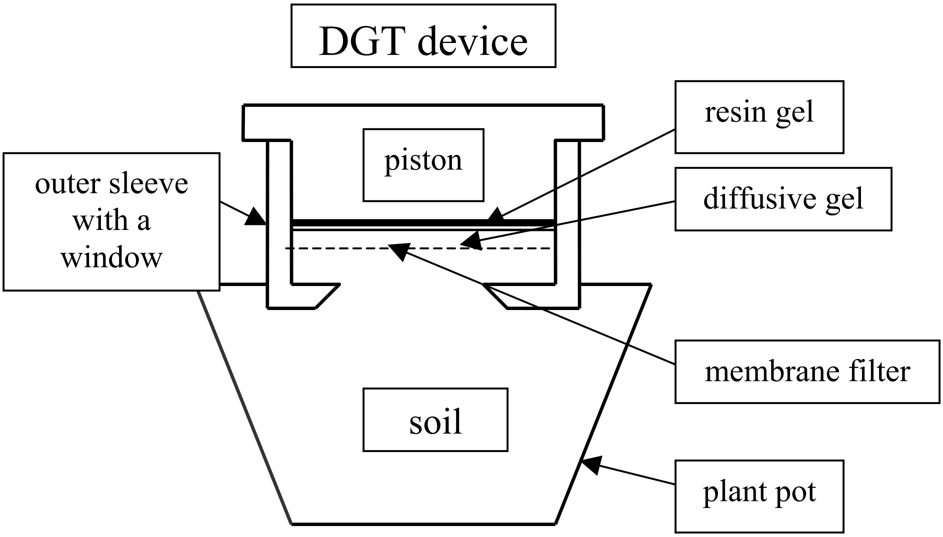

Figure 5.2. Cross section through a DGT device deployed in a pot of soil. For illustrative purposes, the DGT device is shown proportionally larger than the pot. Reprinted with permission from Zhang et al., 2001 (American Chemical Society)

It simply acts as a physical surrogate of plant uptake by interposing a diffusive gel layer between the resin and the soil, through which ions and complexes can diffuse freely. From this, fluxes to the resin and corresponding concentrations can be measured based more on kinetic than equilibrium principles. Like plants, by lowering local concentrations in the soil solution, DGT responds to the *labile metal pool* resupplied from soil solution and solid phases. The measured *DGT metal flux* can be quantitatively related to the effective concentration (C_E), which is directly related to plant metal uptake, and encompasses both soil solution concentration and its enhancement from the solid phase. The DGT concept is based on our understanding of diffusional processes occuring in the rhizosphere. If the C_E can be directly related to plant uptake, it may provide a measure of the potential hazard of metal-contaminated soils. As expected, C_E is largely determined by soil properties and depends on the deployment time and of the thickness of the diffusive gel layer.

Another membrane technique, the ”Helmke-Donnan Membrane Technique” (HDMT) has been developed for the direct measurement of soil solution activities of metal ions. Here, free metal ions are allowed to equilibrate across a negatively charged ion-exchange membrane. Once equilibrium has been established, the solution on the acceptor side of the membrane can be analysed for metals by traditional analytical methods (FAMEST, 2000).

Measurements of C_E of **copper** were made by Zhang et al. (2001) on 29 different soils covering a wide range of concentrations both as EDTA-extractable Cu and as free Cu^{2+} activity, and these levels were compared to Cu concentrations in *Lepidium heterophyllum* (pepperwort) grown on these soils. Here, *L. heterophyllum* was used to lower, like the DGT devise, local soil solution **Cu** concentrations, due to a fast transfer step. This does not disregard other transfer processes where plant uptake is slow, but indicates that for **Cu**, *resupply from solid phases* was supposed to be the controlling step in the soil. In fact, plant Cu was linearily correlated with C_E, but was more scattered and nonlinearly related to the free Cu^{2+} activity, the EDTA-Cu or soil solution concentration. This may indicate that *diffusion* and *labile metal releases* are dominant supply processes, which the DGT-soil system mimics best. Also quantitatively, C_E proves to be an accurate measure of the true Cu bioavailability in these soils (see also section 5.6). At $C_E > 2$ mgCu/L, plant yield decreased sharply on all soils indicating severe Cu phytotoxicity (see also chapter 7). It has been shown by others (cited in Zhang et al. 2001) that DGT-labile species exclude strongly bound organically complexed metal and colloidal species supposed not to be utilized by plants, but include labile solution species, e. g. inorganic and fulvic complexes along with the kinetically labile supply from solid phases.

The authors further discuss if the free ion activity model can be applied when membrane transport is so slow, so that no local depletion of metals occur. From their results they conclude that the *kinetically labile solid phase pool of metals* plays an important role in plant uptake and is part of the DGT measurement. DGT devices may provide a major step forward to assess the potential hazard to plants of metal contaminated soils by means of major geochemical data.

Although being only a small fraction of the dissolved soil metal concentration (e. g. for Cu and Pb between 10-8 and 10-10, and for Cd between 0.002% and 13%; see Allen 2002), evidence is accumulating also in soil research that it is by and large the *free metal ion activity in the soil pore water*, rather than total or dissolved metal concentration, which mainly controls metal bioavailability to soil microorganisms and plants. By determining the soluble Cu and free Cu^{2+} activity in the pore water from 22 soils with total Cu concentrations varying from 19 to 8645 mg/kg, Vulkan et al. (2000) found that the percentage of free Cu^{2+} in the total soluble Cu fraction varied from 0.02 to 96 % and was influenced mainly by pore water pH and less by DOC. For example, at soil pore water pH > 6, free Cu^{2+} was less than 1 %. Porewater samples were also analyzed by two bioluminescence-based bacterial biosensors, i. e. *Escherichia coli* and *Pseudomonas fluorescens*. The response of *P. fluorescens* correlated better with pCu^{2+} than with total soluble Cu, whereas *E. coli* showed a correlation to both Cu forms, with EC_{25} and EC_{50} values of pCu^{2+} for the two biosensors of 5.8 and 5.0, respectively. Previous work also done by the authors showed that biosensors reacted also in the same way to free Zn^{2+}-ions in the pore water of sewage sludge-amended soils (ref. given therein; see section 5.5.7). Although there was a fairly good correlation between soluble Cu and the total soil Cu content, k_d values ranged widely from 515 to 9294 L/kg. Also expected, soil pH and DOC were the main k_d influencing factors, but went in opposite directions, as can be seen from the obtained regression equation: $\log(k_d) = 1.74 + 0.34\ \mathrm{pH} - 0.58 \log(\mathrm{DOC})$.

The equation explained only 42 % of the observed variability of k_d indicating a large uncertainty in the attempt to predict k_d solely from soil properties (pH, DOC). In contrast, variations of Zn (and Cd) were much smaller and soluble Zn mostly < 1.0 mg/l. Also pore water pCu^{2+} was mainly influenced by soil pH and total Cu concentration according to:
$pCu^{2+} = 1.79\ \mathrm{pH} - 1.47 \log(\text{total Cu}) - 0.53$.

McBride and Martinez (2000) investigated the speciation of Cu in a highly Cu-contaminated soil (3000 mg/kg dw) after amemdment with metal-adsorptive materials (see also section 5.5.7 below). Total soluble (measured by ICP-MS) and labile (measured by ASV) Cu concentrations partly decreased, but none of the amendments was able to reduce soil phytotoxicity as indicated by bioassays. How intricate the situation can be, was shown by the addition of Mn oxide (birnessite), which on one hand lowered the free Cu^{2+} concentration, but on the other hand phytotoxicity persisted, probably due to an increase in DOC and hence of soluble and labile Cu forms. The toxicity of Cu to maize shoots appeared to depend on labile as well as free ionic Cu forms. The intricate and dynamic interrelationship existing among *free, labile and nonlabile pools* of Cu in soils is illustrated in Figure 5.3. The authors conclude that due to the large size of the labile pool relative to free Cu^{2+}, and the even larger Cu reserve in soluble nonlabile forms and soil solids, the free Cu^{2+} activitiy may be very well buffered. This would explain the observed lack of reduction of the Cu^{2+} activity upon treating the soil with various amending materials.

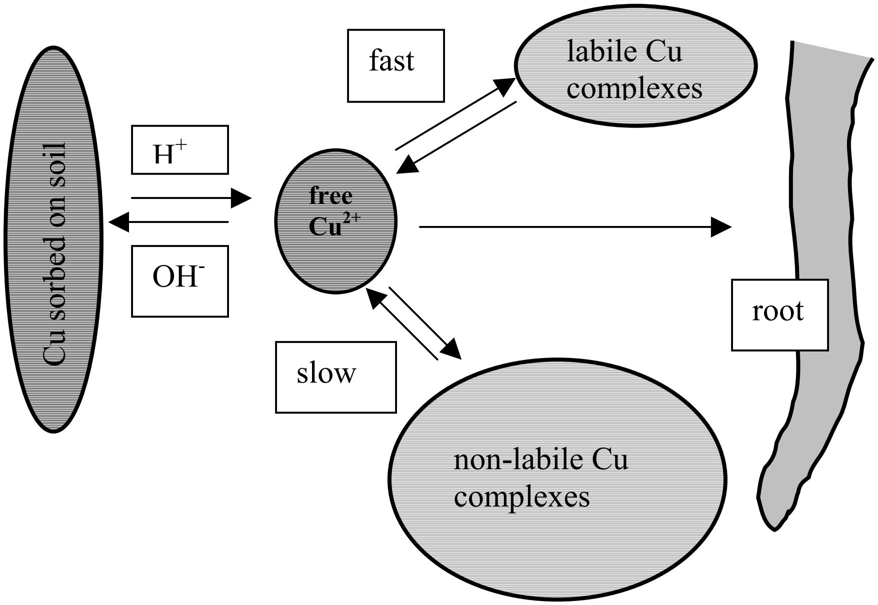

Figure 5.3. Schematic representation of the dynamic interactions between free, labile and nonlabile metal pools in soil solution and their uptake by plant roots Reprinted with permission from McBride and Martinez, 2000 (American Chemical Society)

5.5.4 Surface reactions

Although representing only a small fraction of the total soil mass, colloids or coatings constitute a significant fraction of the surface available for interactions between soil particulates and dissolved metals. Surface complexation modelling is the method of choice today to mimic, predict and understand also acid/base and metal binding properties of oxide surfaces in soils.

It is well known that Fe oxides and organic matter coexist in soils and serve together as a reactive adsorbent pool for trace metals, due to their high surface area and high reactivity associated with a variety of functional groups. Both groups of soil compounds are intimately associated as oxide-organic complexes making it often difficult to discern their individual roles. In general, the tendency of metals to react with mineral and organic *components limits their solubility and hence bioavailability and toxicity. This type of solubility control is attributed to various surface-related mechanisms like cation exchange, specific adsorption to surface hydroxyl groups, co-precipitation, and multinuclear complex formation at the surface, or precipitation of discrete oxides or hydroxides. Organic matter contains various S-, O- and N-functional groups that strongly bind metals; hence, it can even act as a better adsorbent than Fe oxides and, thus, decrease metal bioavailability.*

Sorption rates on cell walls vary due to the large variety of chemical functional groups on the cell surface, which create a negatively charged surface. Also here, spectroscopic methods, like XAS (X-ray absorption spectroscopy) can be used to determine the chemical nature and the structure of different binding sites on the cell surface. In combination with solution chemistry, spectroscopic studies suggest that there may be a large number of mixed metal-ligand complexes with intermediate affinities between high and low affinity sites.

Although representing only a small fraction of the total mass of soil, *inorganic and organic colloids or coatings* constitute a significant fraction of the surface available for interactions between soil solids and metals in solution. Robertson and Leckie (1998) used *surface complexation modelling* to mimic, predict and understand acid/base and metal binding properties of oxide surfaces. The obtained experimental adsorption data suggested the existence of *multiple classes of* ***Cu*** *binding sites*. In addition, crystallographic analysis indicated several types of surface hydroxyls on the crystal faces of goethite (α-FeOOH). From this, the authors summarize that Cu partitioning on goethite (as an example) is strongly pH-dependent but not consistent with a single-plane, single-site surface class system. The true situation is better described by assuming a second high-affinity Cu binding site with a low surface density suggesting sites located at steps, kinks etc. on the mineral surface (compare to section 5.4.7.2).

Also Morton et al. (2000) investigated the *adsorption kinetics* between **Cu** and surface hydroxyl sites on soil oxides and clay minerals. They assume that transition metals in general form strong *inner-sphere complexes* on these sites. It was shown that Cu, sorbed as surface complex on montmorillonite, γ-alumina, ferrihydrate and geothite, was bioavailable. But with increasing concentration of surface sites, the bioavailability of Cu (expressed by the methane monooxygenase (MMO) activity in the methanotroph *Methylosinus trichosporium OB3b*) decreased again. In this context, still not much is known (in contrast to sediments) about how living cells bind and sequester trace metals in soils. As an example, a protein (the so-called ***Cu** binding compound/cofactor CBC*) with a strong Cu affinity, was found for methanotrophs (i. e. bacteria using methane as energy source). This protein seems to sequester Cu in a *siderophore-like way*, by which the metal binding ligand is excreted during cell growth and subsequently reinternalized. Due to its ability to sequester extracellular Cu, CBC is supposed to be a key factor in determining the bioavailability of different Cu forms in soils (see also chapter 6).

It is well known that *Fe oxides and organic matter coexist* in soils and serve together as a reactive adsorbent pool for trace metals, due to their high surface area and their high reactivity associated with a variety of functional groups. Both groups of compounds are intimately associated as oxide-organic complexes making it often difficult to discern their individual roles (Martinez and McBride 1999). Generally spoken, the tendency of metals to react with mineral and organic components limits their solubility and hence bioavailability and toxicity. This type of solubility control is attributed to various *surface-related mechanisms* like cation exchange, specific adsorption to surface hydroxyl groups, coprecipitation, and multinuclear complex or mixed-cation hydroxide complex formation at the surface at higher loadings and pH, or precipitation of discrete oxides or hydroxides. *Metal retention* by ferric hydroxide has been found to decrease in the order: Pb ≥ **Cu** >> **Zn** > Cd. Martinez and McBride (1999) showed that even when metals coprecipitate with Fe (hydr)oxides and are aged for a long time, residual solubilities for Cd, Cu and Zn remain still high enough to reach phytotoxicity or excessive plant uptake. Organic matter contains various S-, O- and N-functional groups that strongly bind metals. In their study, Martinez and McBride used X-ray absorption spectroscopy (XAS) and electron spin resonance (ESR) to demonstrate that Pb, Cu and Zn form inner-sphere complexes with soil humic substances, and that these metals coordinate with oxygen ligands, as well as with S-functional groups, as far as Zn is concerned.

The observed increasing availability of metals in sewage sludge can be chiefly related to the *decomposition of organic matter* (see section 5.5.7 below). Soluble organic ligands resulting from the decomposition of organic matter, change metal speciation in solution and so their bioavailability and toxicity. They may coat reactive mineral adsorption site, inactivating them and therefor inhibit metal adsorption, but in turn sorb on mineral surfaces and generate new sorbing sites and increase metal retention. However, there is evidence that metal extractability and bioavailability remain constant after sludge application is terminated. In a study cited by Martinez and McBride (1999), it was shown that organic matter can even act as a better adsorbent as goethite at pH values between 5 and 5.5, in removing **Cu** from solution. It was further found by others (also cited by Martinez and McBride, 1999) that mineral-bound organic matter enhances the adsorption of **Cu** and Co at pH values < 6.5, and so decreases their solubility. However, at neutral pH values, more mineral components control metal solubility, while organic matter increases metal dissolution by forming soluble metal-organic complexes. Due to competition among multiple and mixed solid phases, it is very often difficult to asses what soil component is responsible for the long-term solubility control of metals in soils. In their study Martinez and McBride (1999) showed that the organic adsorbent they used (i. e. leaf compost) was very efficient in removing **Zn** and Cd.

Another interesting approach to tackle the interaction between dissolved metals and biota (living cells) has been reported by Sarret et al. (1998), who studied Zn and Pb binding sites on the mold *Penicillium chrysogonium* by means of sorption experiments (isotherms) combined with *EXAFS spectroscopy* (see section 5.4.5). It is well recognized today that *sorption rates* on cell walls vary due to the large variety of functional groups on the cell surface, such as carboxyl (–COOH), aldehyde (–COH), hydroxyl (–CHOH), sulfhydryl (–SH), phosphoryl ($–PO_4H_3$), or amine ($–NH_2$), which create a negatively charged surface. To determine the chemical nature and the structure of different binding sites on cell walls, the authors used 'X-ray absorption spectroscopy' (XAS), which has been increasingly used over the last 15 years to determine crystal chemical forms of metals in soils and sediments. Only recently these spectroscopic methods are also used to assess environmentally relevant metal species. By lowering detection limits by a factor of 5-10 below previous levels, these techniques can now help, according to the authors, to identify the nature of even the strongest complexing functional groups on algal cell walls, which otherwise would have been masked by the predominant contribution of high-density, low affinity sites. This was an *"instrumental breakthrough"* and adsorption isotherms obtained by Sarret et al. (1998) indicate that cell walls own at least two adsorption sites differing by concentration and affinity to **Zn** and Pb. The most abundant sites (95%) show a greater affinity for Zn and a lower one for Pb, whereas the less abundant sites reveal a low Zn affinity but a higher for Pb.

At high initial $[Me^{2+}]$ concentrations, resulting *adsorption isotherms* for Zn and Pb converged to a constant $[Me^{2+}]_{ads}$ value (C_{tot}), indicating that cell surfaces conform to the Langmuir sorption type, i. e. sorbed cations do not sorb as a metal hydroxide. EXAFS analysis showed the existence of *predominant reactive surface functional groups on the fungal cell walls* to be similar for Zn and Pb (with about 95% phosphoryl and about 5% carboxyl groups), but with a reversed affinity (see further below). EXAFS indicated that the *low affinity Zn-sites* are chemically identical to the *high affinity Pb-*

sites. In addition, Zn and Pb adsorption isotherms differed at low Me^{2+} concentrations. It was further uncovered that Zn was overwhelmingly complexed by phosphoryl groups in a tetrahedral coordination from low to high concentrations, and to carboxyl groups close to saturation of the reactive surface sites, which is consistent with the interpretation of the adsorption isotherms. The *high affinity Zn1 sites* correspond to phosphoryl groups, while the Zn2 sites are supposed to be composed of carboxyl-Zn complexes with a lower stability formed at high Zn concentrations. Based on solution chemistry and the obtained EXAFS results, there seems to be a large number of mixed phosphoryl-Zn-carboxyl complexes of intermediate affinity between these 2 sites (Zn1 and Zn2), as also described thermodynamically by a *continuous distribution of affinities*. The performed EXAFS analysis also allowed to demonstrate that Pb was predominantly bound to cell walls by carboxyl (high affinity Pb1 sites) groups at very low concentrations and to phosphoryl (Pb2) at high concentrations. Summarizing the results for Pb, the authors explain that the minor COOH-surface ligands (5%) of the *P. chrysogenum* cell walls have a high affinity to Pb compared to the predominant PO_4 ligands (95%). For this reason, Pb-COO complexes are preferentially formed at low Pb conccentrations, and the predominant PO_4-Pb complexes at high Pb concentrations. These binding characteristics are also confirmed by the presence of *2 distinct plateaus on the adsorption isotherm*, but are, as mentioned before, in contrast to the binding charcteristics for Zn, where COOH ligands reveal a relatively low affinity.

5.5.5 Redox effects

Oxidation-reduction reactions in soils, e.g. as a result of soil flooding or drainage, may not only affect the speciation of red-ox-active metals, like Cr or Mo, but also of red-ox-stable metals, like Zn, Cu or Ni. Reductive dissolution of Fe and Mn hydroxides under sub-oxic conditions may release these trace metals into soil pore-water, while persistence of sub-oxic or anoxic conditions may lead to their subsequent partitioning into sulphides or carbonates.

Dissolved metal concentrations in terrestrial systems also vary significantly with seasonal changes, which are triggered by concomitant light and temperature variations, which in turn affect the activity of microbes. In fact, the release of metals from soil particulates often coincides with a decline in red-ox potential and an increase of organic carbon in the soil solution, induced by microbial activity.

Acidic sulphate-rich soils are formed as a result of the oxidation of anoxic, sulphide-rich soils, which may be caused by human (e.g. digging) or natural activities (e.g. land-rise). The production of sulphuric acid and subsequent Fe precipitation significantly changes natural soil characteristics. Acid soil conditions cause the release of metals from various soil minerals and contribute to increased concentrations of metals, like Cd, Ni, Zn and Cu, in soil run-off and receiving surface waters.

Like in sediments (compare section 5.4.9), redox potential is one of the critical factors regulating the speciation and bioavailability of metals in soils. In the following, some examples from the most recent literature are given to document some new results. Oxidation-reduction reactions may not only affect the partitioning of redox-active trace metals, like **Cr or Mo**, but also of redox stable metals like **Zn, Cu or Ni**, in soil or aquatic environments. For example, the reductive dissolution of Fe and Mn hydroxides under suboxic conditions may release redox-stable trace metals into the aqueous phase, whereas persistence of suboxic or anoxic conditions may lead to their partitioning into sulphides or carbonates. But slow transformation rates and fluctuations in physicochemical conditions can alter the predicted species changes.

Bostick et al. (2001) determined the speciation of **Zn** within a seasonally saturated mining-impacted wetland soil by using XAS (see section 5.4.5), in relation to the soil redox status. Each Zn-containing species was spectroscopically identified by its unique set of interatomic distances and their presence evaluated by comparing the bond distances found in the soil sample with those of model compounds (compare to Table 5.8 in section 5.4.5). The XAS data suggested at least the presence of four primary Zn species within these wetland soils, namely ZnO, $ZnCO_3$, Zn sorbed to hydroxide, and ZnS. Zn speciation seemed to be strongly correlated with the soil waterdepth. In dry oxidized areas almost all Zn was sorbed on hydroxides or present as discrete ZnO phase. On the other hand, ZnS and $ZnCO_3$ was formed in response to a lowered redox potential associated with soil flooding. In soils under intermediate suboxic shallow water conditions, Zn occurred as ZnO, sorbed or as ZnS. Thus waterdepth and redox status appeared to be the most important variables for the speciation of Zn in these wetlands.

To verify the effects of depth, duplicate soil cores were taken from various sites within the wetland (see Table 5.14 below). The cores collected at different sites contained different Zn species, even when their water level was similar. But despite these differences, Zn was mainly determined by the redox stability of each particular phase. Therefor, in oxic soil cores, either ZnO or Zn adsorbed onto hydroxides were dominant, whereas in anoxic environments, ZnS or $ZnCO_3$ dominated, indicating that the water level and corresponding redox status were indeed most relevant for Zn speciation in these wetland soils.

Table 5.14. Influence of water depth and redox status on Zn speciation in wetland soils (from Bostick et al., 2001)

Water depth (cm)	core section (cm)	% ZnO	% sorbed Zn on hydroxide	% ZnS	% $ZnCO_3$
0	0-5	0 ± 0[a]	97 ± 2	3 ± 2	0
0	5-10	0 ± 0	96 ± 2	4 ± 2	0
36	0-5	0 ± 0	38 ± 2	62 ± 2	0
36	5-10	0 ± 0	36 ± 4	64 ± 4	0
39	0-5	43 ± 2	0 ± 0	57 ± 3	0
39	5-10	43 ± 5	0 ± 0	57 ± 5	0
89	0-5	0	0	85	15
89	5-10	0	0	80	20
105	0-5	12	0	0	88
105	5-10	0	0	0	90

[a] each value represents the average of duplicate cores, ± standard deviation. Where single values are given, only one core was analysed

Otero (2002) investigated the occurrence of metal sulphides in salt marsh soils in NW Spain and the interaction between seasonal and spatial variations and the Fe and S redox cycle. In high salt marsh soils (with suboxic redox conditions), iron sulphides were low (both as AVS and pyrite) indicating a low degree of trace metal *pyritization* (DTMP) in surface layers. However, metals associated with the pyrite fraction increased with soil depth. In turn, low salt marsh soils (anoxic conditions) showed maximum metal sulphide contents already at the soil surface, which was colonized by *Spartina maritima*. It is known that the roots of *S. maritima* stimulate the activitiy of SO_4-reducing bacteria in strongly reduced soils, but also favour partial soil oxidation and so the formation of polysulphides, which may immediately precipitate with Fe^{2+} as pyrite. In the deep permanently anoxic layers, pyrites form in a reaction with FeS probably as an intermediate, according to: $FeS + H_2S = FeS_2 + H_2O$. The authors observed also seasonal variations in metal sulphide concentrations with lowest levels in summer due to a net loss of metal sulphides (e. g. by evapotranspiration of H_2S and release of oxygen from plant roots). In contrast, highest concentrations of AVS and pyritic metals occurred in summer in the low marsh soils, which were colonized by vascular plants with an increased activitiy of sulfur-reducing bacteria, resulting in an accumulation of soil metal sulphides, probably also due to the higher ambient temperature.

Soils not colonized by plants had highest pH values and lowest porewater metal concentrations during summer, suggesting that the higher temperature increased the activitiy of SO_4-reducing bacteria, leading to increasing alkalinity and sulphide concentrations in the porewater. Under these conditions metals are precipitated and removed from porewater, increasing AVS and pyrite in soil. In contrast, sulphide oxidation in soils colonized by *Spartina maritima*, showed at the same time a decrease in pH and a concomitant release of trace metals in the porewater (Otero and Macias 2002). Although comparatively low (only 0.43% of pyrite-Fe and 1.38% of AVS-Fe, and trace metals Cu, Ni, Zn, Mn and Cr only about 30-1000 times lower), measured metal porewater concentrations were consistent with metal concentrations in the pyrite, but not in the AVS (1 N HCl) or total metal fraction. From their field studies, the authors suggest that *pyrite oxidation* may be a final step in the precipitation of authigenic Fe oxide minerals, an assumption which may be confirmed by the observed high Fe oxyhydroxide concentrations in the upper soil layers, a process removing again dissolved metals from the porewater.

Quantin et al. (2001) studied the *bacterial reduction of Fe and Mn oxides* in a Ferrasol by laboratory batch experiments. They found that the anaerobic Fe and Mn reducing bacterial activity (measured as anaerobic respiration or fermentation) in that soil was responsible for the observed Fe and Mn oxide solubilization. Oxide reduction increased when more organic C (as electron-donator) was available. It was also evident that Mn oxide was the major reducible phase and metal source before goethite. Both **Ni** and Co solubilized with Fe and Mn oxides, but decreased again at the end of the experiment, probably due to adsorption or precipitation processes.

Fortin et al. (2002) assessed the role of *SO_4-reducing bacteria* (SRB) on the fate of Fe and SO_4 in **Cu-Zn** and Au tailings by combining SRB analysis, solid-phase mineralogy, and porewater geochemistry. The oxidized surface of the Cu/Zn tailings showed a low pH, high redox potential and high Cu, Zn and SO_4 porewater concentrations, and depletion of pyrite. In contrast, Au tailings revealed a more neutral pH, slightly anoxic conditions, low Fe and porewater metal concentrations and little pyrite oxidation. The authors assume that SRB are involved in SO_4 reduction occuring in the Cu/Zn tailings because SO_4 solubility was not controlled by SO_4-rich minerals. On the other hands, in the reduced tailings zone the occurrence of soluble Fe indicated the presence of *iron reducing bacteria* (IRB). The observed SO_4 decline and release of soluble Fe into porewaters was paralleled by an increase in pH and alkalinity. In contrast to the Cu/Zn tailings, SO_4 and Fe solubility in the Au tailings was supposed to be partly controlled by jarosite (a K-Fe-SO_4-OH mineral) and Fe oxides.

Dissolved metal concentrations in terrestrial systems vary significantly with seasonal changes, which are triggered by changing light and temperature conditions. Olivie-Lauquet et al. (2001) observed marked *seasonal variations* for soluble metals in soils, except for **Zn and Cu**. Release of metals from soil particulates coincided with a decline in redox potential and an increase of organic carbon in the soil solution. The decline in E_h was inversely correlated with ambient temperature, dissolved Fe and Mn, and organic carbon. The authors suggest that microorganisms using soil Fe and Mn oxyhydroxides as e-acceptors catalyse redox changes and induce the observed increase of dissolved organic carbon.

Acid sulfate soils are a rather widespread phenomenon in various parts of the world (e. g. in Scandinavia, Northern America) and mainly the result of the oxidation of O_2-free, sulphide-rich soils due to human (e. g. digging) or natural activities (e. g. landrise). The production of sulfuric acid and subsequent Fe precipitation significantly can change natural soil characteristics. As an example, in Sweden, acid sulfate soils, which are used for agrocultural purposes, occur, according to Sohlenius and Öborn (2000), by and large along the northern part of the Swedish Baltic Sea coast, and around the big lakes Mälaren and Hjälmaren. Resulting acid soil conditions are releasing metals from various soil minerals and cause increased concentrations of metals, like Cd, Ni, Zn and Cu in surface waters draining these acid sulfate-rich soils.

5.5.6 Aging and weathering

Effects of soil aging on trace metal fluxes can be the enhanced retention via sorption, precipitation/co-precipitation, occlusion or incorporation into reservoir minerals, leading to stronger metal adsorption and decreasing metal extractability. Metal sorption can become irreversible due to long term diffusion into the crystal lattice of reservoir minerals, like goethite. In addition to irreversible adsorption, diffusion through pores in the organic matter or pores coated with organic material may slow down metal desorption. Indeed, most empirical studies show a higher portion of mobile metal fractions in recently contaminated soils than in old ("aged") contaminated or unpolluted soils.

Also weathering processes, like the formation of secondary minerals or the degradation/mineralization of organic compounds, have profound effects on metal mobility and bioavailability in soils. However, aging and weathering act differently for different metal forms. For example, Fe hydroxides known as an important sink for long-term metal retention, may sometimes fail to reduce activities of trace metals in the soil solution, to reach permitted metal loadings (e.g. for sludge application).

Regarding the bioavailability and extractability of persistent organic contaminants, we know that both will decrease in general with increasing *soil-pollutant contact time*. In how far this may be also valid for trace metals in terrestrial systems is still not very well established (see also following section). *Effects of aging* are described as enhanced retention via sorption, precipitation/coprecipitation processes, occlusion and incorporation into *reservoir minerals*. For instance, it is experimentally observed that metal sorption is irreversible due to long term diffusion into the crystal lattice of e. g. goethite. In addition to *irreversible adsorption*, diffusion through pores in organic matter or pores coated with organic material may cause the observed slow desorption (Ahlf and Förstner 2001). Two recent review paper provide an excellent background of processes inherent to aging, and possible consequences on metal bioavailability/toxicity and appropriate extraction techniques (by Reid et al., 2000; McLaughlin et al., 2002). Most empirical studies show indeed that mobile metal fractions in recently contaminated soils are frequently higher than in old contaminated or in unpolluted soils. Without a model addressing the ”aging” of metals, risk assessment can deal only with soils that have been polluted for a long time, and hence have reached an equilibrium (McGrath, 2002).

Natural weathering processes in soils, like the formation of secondary minerals or organic degradation/mineralization, have a profound effect on the mobility and bioavailability of trace metals. Fujikawa et al. (2000) observed a downward movement of **Cr and Ni** (and Fe and Mn) in a natural soil probably induced by long-term weathering processes. Highest water extractability was obtained for Mn, **Zn** and Ba, whereas extractability of Zn and Pb in near-surface soil layers was higher than in deeper layers, indicating weathering and aging effects.

Schaaf et al. (1999) pointed out that the development of a scientifically sound soil solution chemistry in different mine soils (lignite and pyrite substrates) needs a clear knowledge of processes like the weathering of primary and the formation of secondary minerals, and of the size of the acid production and buffering capacity. Soil solution analyses showed that pyrite oxidation occurred in these soils causing the observed extremely low pH values, and high $Fe^{(n+)}$ and $SO_4^{(2-)}$ levels. The strong acid production accelerated the weathering of Al silicates and caused high Al levels in the solution of the soil. In addition, rather low leaching rates enabled the formation of secondary phases, which in turn control the composition of the soil solution.

Martinez and McBride (1998) presented investigations on the solubility of trace metals after simultaneous coprecipitation with Fe (hydr)oxides and after long-time aging of the precipitate. Controls on soluble metals by clays and oxides could be attributed to ion-exchange (*non-specific adsorption*) and surface complexation (*specific adsorption*) to hydroxyl groups, coprecipitation (*solid solution*, i. e. isolated atoms within a large oxide structure), and *precipitation* as discrete oxides or hydroxides. According to the authors, our current knowledge on processes involved in metal retention obtained so far from adsorption analysis and solution chemistry, or by spectroscopic and microscopic techniques, suggests a *'continuum'*, formed when metals adsorb to clays and oxides, extending from specific adsorption to precipitation. 'Extended X-ray absorption fine structure analysis' (EXAFS) demonstrated the direct adsorption of metal ions (Cd^{2+}) onto the surface of hydrous ferric oxides and α-FeOOH as mononuclear surface complex. But also multinuclear surface complexes were evidenced by the performed X-ray absorption studies (XAS). Formation of a surface precipitate from Ni^{2+} sorption onto pyrophyllite was shown by transmission electron microscopy (TEM). Subsequent XAFS analysis indicated the formation of a bidentate inner-sphere complex and of a mixed Ni-Al-hydroxide on the clay and on the Al-hydroxide surface. The use of 'Electrophoretic Mobility' (EM), 'Electron Spin Resonance' (ESR) and 'Scanning Force Microscopy' studies indicated the coexistence of adsorbed metals at isolated sites on oxide surfaces (e. g. of Cu^{2+}, Mg^{2+}, Mn^{2+}), the formation of 'surface clusters' (for Cr^{3+}) at isolated areas, but also of evenly distributed precipitates (for Cr^{3+}) on the surface (of geothite). These different surface morphologies resulted in the observed different extractabilities (by oxalate).

Both the *increased metal adsorption* and *decreased extractabilities with time* (aging) may be explained by these surface phenomena rather than by solid-state diffusion processes. From this it seems that the *metal sorption 'continuum'* begins with specific adsorption at discrete sites with multinuclear complexes or mixed cation hydroxide complexes forming at the surface at high metal loadings. These complexes may distribute evenly over the surface or may form clusters.

More intriguing, different metal forms may coexist and their solubility determined by their chemical and morphological/structural characteristics, will vary with time. *Surface complexation constants* for high affinity binding sites on hydrous ferric oxide can be calculated from adsorption experiments and are based on the reaction: $FeOH^0 + M^{2+} \leftrightarrows FeOM^+ + H^+$, where $FeOH^0$ is a high affinity binding site on the oxide surface, and M^{2+} a divalent cation. The sorption experiments by Martinez and McBride (1998) gave log K_1int [*]of 0.47 for Cd^{2+}, 0.99 for Zn^{2+}, 2.89 for Cu^{2+} and 4.65 for Pb^{2+}. From this the solubility of metals decreases in the order: $Cd^{2+} > Zn^{2+} > Cu^{2+} > Pb^{2+}$. Solubility lines drawn for discrete Cu precipitates (hydroxide, carbonate, oxide) showed a higher solubility than for coprecipitates. Franklinite had the lowest solubility of all Zn species. The solubility of Cu and Zn as calculated from the intrinsic surface complexation constants for hydrous ferric oxide (as taken from literature data) predicted a lower solubility than was found for the amporphous coprecipitates established here.

According to thermodynamic principles it is suppposed that *coprecipitation* ("true" solid solution) results in the lowest solubility. However, recent spectroscopic and microscopic evidence suggests that formation of multi-nuclear and mixed-metal phases at mineral surfaces are more common and that adsorption at discrete sites ("coprecipitation") may be less prevailing than previously thought. Consequently, so the authors conclude, metal solubilities and hence their bioavailability calculated from surface complexation models may underestimate metal solubilities in real systems. The data presented by Martinez and McBride may indeed not indicate discrete-site adsorption or formation of true solid solutions at least under the experimental conditions. Instead, *polymerization* of Cu^{2+} hydroxy species (and to a lesser extent of Zn^{2+}), and their segregation/adsorption within/on the Fe hydr(oxide) may explain the observed phenomena. The adsorbed metal hydroxy polymer may have a higher solubility than the metal adsorbed to isolated sites. Also pH changes have to be considered as important factor controlling the observed metal solubility behaviour.

When the *soil metal retention capacity* is overloaded, or when metals are solubilized, e. g. at low pH, a significant downward transport of metals from the soil surface occurs. Metals will travel downward with the leaching water. Little attention has been given in the examined literature to the actual concentration remaining in soil solution after pH has changed. These concentrations can still be high enough to be toxic to biological systems. The results presented by Martinez and McBride (1998) show that metals coprecipitating with Fe hydroxides can have solubilities, which result in free

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[*] K_1int = intrinsic surface complexation constants

metal ions (Cd, Cu and Zn) that are toxic to sensitive crops (shown in hydroponic experiments; references given), like alfalfa (pZn = 5.0-5.4) or wheat (pCu = 8.2). In summarizing their results, the authors conclude that although Fe hydroxides may be an important sink for long-term metal retention and have been successfully used to remediate metal-contaminated soils, they may fail to lower activities of some trace metals, including Cu, to reach the U. S. EPA permitted metal loadings (e. g. for sludge aplication).

5.5.7 Sludge application and contaminated soils

To maintain sustainability, recycling of plant nutrients released from urban activities back to the agricultural system is needed. The use of sewage sludge on agricultural soils is regulated by EU directives as well as national legislation. Limit values for trace metals in sewage sludge are still expressed as total concentrations per unit of dry weight sludge. However, when assessing the environmental and health risks associated with sludge application, we need to know the form in which a metal is introduced with the sludge into soils, and how this will change depending on prevailing soil characteristics.

For example, water-soluble Cu in the soil solution seems not be an appropriate measure for bioavailable Cu, as most of this metal in soils occurs as non-bioavailable organically complexed. The free cupric ion (Cu^{2+}) *activity, on the other hand, is supposed to be a more appropriate parameter, but still to cumbersome to monitor in a routine way. Recent investigations on Zn suggest that maximum sludge loadings should be based on metal amounts in the soil solution rather than on total input or total contents in the soil mass.*

It has been shown that metal concentrations in soil and crops increase during the first years after sludge application, while organic matter rapidly decreases. Beside pH, also temperature proved to be another critical soil parameter, with higher plant and soil metal concentrations at higher temperatures, probably due to increasing organic decomposition rates. Extractability and availability of metals increases in experimentally treated soils, due to organic matter decomposition, at application rates similar to those applied in common agricultural practice (8-10 t/ha), especially under warmer conditions. Recent studies also confirm previous observations that sludge-borne metals added to soils are less strongly bound to particle surfaces than indigenous metals found in the soil, before sludge addition. Organic degradation leads to the break-down of humic macro-molecules *and the formation of more labile metal forms and soluble organo-metallic complexes with low molecular weight organic ligands (DOC), which are easier for plants to absorb (from soil solution or loosely bound onto soil surfaces). Although sludge application may increase the transport of metals (and DOC) in the dissolved form, often no significant increase in total soil metal contents is observed*

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To maintain a sustainable agriculture and society, the recycling of nutrients from the city back to agricultural lands is needed. Due to its *high nutrient content* and *soil ameliorating properties*, sewage sludge from wastewater treatments is an essential part of this recycling process. The problem is that it contains also potentially toxic elements, like trace metals.

The EU Directive 86/278/EEC regulates the use of sewage sludge on agricultural soils. According to this Directive, the permitted trace metal loads per year, calculated as a 10-year average are: for Cu – 12 kg/ha, for Ni – 3 kg/ha, and for Zn – 30 kg/ha. However, in a recent EU initiative (i. e. the Third Draft Working Document on the Revision to the Sewage Sludge Directive), the Sewage Sludge Directive has been revised, and in the case of Cu, the *annual permitted application rate* on agricultural soils is proposed to be 3 kg/ha in the near future. Many of the EU Member States are already using much lower limit values; e.g. in Sweden, the allowed annual application rates to agricultural soils are set at 0.3 kg Cu/ha, 0.025 kg Ni/ha and 0.6 kg Zn/ha. On the other hand, permitted trace metal loads to soils in Canada and the USA are still much higher (compiled in Landner et al., 2000; cf Table 4.6).

Most EU Member States have expressed *limits* for Cu, Cr, Ni, Zn and other trace metals in sewage sludge in terms of total concentrations per unit of dry weight. In a critical review, Landner et al. (2000) evaluated Cu concentrations and loadings reported in the relevant literature for soils that do not harm the soil ecosystem. With regard to elevated Cu concentrations (mg/kg dry weight), the microbial community has been found to be the most vulnerable target in soil systems. Notably *nitrification* and *denitrification* seem most sensible. *Lowest observed no-effect concentration* in field soils were reported to be about 5 times higher than in laboratory bioassays with a range of 25-35 mg/kg dw of total Cu (as threshold concentration for the most sensitive microbial soil systems), and about 3-4 times the original background. Invertebrates seem to tolerate 30-40 mg/kg dw. Toxicity threshold values for plants are mostly given not as total Cu concentration, but as the bioavailable part, which was very low depending on various soil factors. *Critical total Cu levels in soils* obtained from the literature are supposed to vary around 50-60 mg/kg dw, while *European limit values* established for agricultural soils receiving sewage sludge are between 36-60 mg/kg dw (in the newest draft, limit values range from 40 to 100 mg/kg).

McGrath (1994) reported experiments confirming a *decreasing N-fixation in soils* treated by metal-contaminated sludge. Nitrogenase activity decreased by 50% in soils containing the metal concentrations depicted in Table 5.15. In field experiments in Germany, sludges were applied for almost 10 years to an old arable and ex-woodland soil with the objective to reach the German limits for metal concentration in soils receiving sewage sludge. Comparing the obtained soil levels with limit values reveals that most investigated metals remain in the same order of magnitude as established limits, but partly exceed, in particular Zn and Cu, the 50% nitrification inhibition value.

Also in this context it has been shown that *water-soluble Cu* is not an appropriate measure for bioavailable Cu as most of it occurs as non-bioavailable organic complex. The *free cupric ion (Cu^{2+}) activity* on the other hand has been supposed as a more appropriate criteria, but method and calculation are still cumbersome (according to Landner et al. 2000). For this reason toxicity threshold values are still related to *plant tissue concentrations*. For Cu it is supposed that a 10 % growth reduction for sensitive plants may occur at a plant tissue concentration between 10-30 mg/kg dw (usually measured in shoots).

Table 5.15. Comparison of metal concentrations in soils of two field experiments in Braunschweig/Germany that received 100 or 300 m^3 $year^{-1}$ of metal-contaminated sewage sludge for about 9 years (Germany), with metal concentrations in soils from them Woburn Market Garden Experiment/UK giving 50% inhibition of nitrogenase activity by cyanobacteria, in relation to German, UK and CEC metal limits for soils receiving sewage sludge (mg/kg dw) (after McGrath, 1994)

Element	Arable field pH 6.1-6.8	Ex-woodland pH 5.3-5.7	50% inhibition of nitrification	German (1982)	UK (1989) (SI 1263)	CEC (1986)[a]
Zinc	160-380	160-410	110	300	300	150-300
Copper	40-100	40-110	30	100	135	50-140
Nickel	10-25	15-30	20	50	75	30-75
Cadmium	1-3	1-3	3	3	3	1-3
Chromium	40-90	40-110	80	100	400	100-150
Lead	60-90	50-100	40	100	300	50-300

Source: Sauerbeck and Rietz (personal communication). [a]for soils of pH 6-7

Reviewing the relevant literature showed that Cu-containing sludge applied to soils does mainly affect soil microorganisms (e. g. N-fixation), but not crops (see Landner et al. 2000). For example, denitrification was hampered at a loading equivalent to the EC-Directive value of 12 kg/ha/year. In contrast, Cu loadings from sludge leading to Cu soil concentrations 55 times the original level in natural soils did not reduce crop yields, after sludge application, indicating that Cu in sewage sludge may be in a rather nonbioavailable form.

Adverse effects on nitrogen fixation have been observed with the help of N^{15}-dilution experiments in soils amended with heavy metal contaminated sewage sludge (Obbard and Jones 2001). For **Zn**, Cela and Sumner (2002) studied threshold values in soil that prevent inhibition of nitrification. Potentially bioavailable Zn was evaluated by sequential chemical extraction. The water-extractable Zn fraction proved to correlate with nitrification inhibition limits. Three *nitrification inhibition ranges* have been identified: 0-0.125 mg/l Zn (no inhibition), 0.125-0.5 mg/l (partial inhibition), and > 0.5 mg/l (complete inhibition). For the same Zn loading, water-soluble Zn was much lower in biosolid-amended soils than in Zn-spiked soils. The authors suggest that maximum Zn loadings should be based on metal amounts in the soil solution rather than on total input or total contents.

Cu balances based on recent literature data showed that this metal accumulates in the top soil layer after repeated sludge application over years, and that we still lack expertise on the *long-term mobility* of Cu from this pool, and on possible risks to contaminate groundwaters (Landner et al. 2000). However the data also show that copper applied as cupric sulfate or Cu-rich fertilizer to Cu-deficient soils may result in even higher Cu inputs than by only sewage sludge addition. *Cu deficiency* is supposed to develop at 5-8 mg/kg dw. For this reason, in Sweden (as an example) about 34 t/year of Cu are currently applied to arable land to counteract Cu deficiency, in addition to 52 t/year, which are added to animal feed and subsequently spread as manure on fields, in comparison to the 28 t/year of Cu that is applied by sewage sludge. This causes the question, if sludge-Cu may become bioavailable in the long-term, and how much of the otherwise added Cu it may be able to replace (Landner et al. 2000).

Like Cu, also Zn enters the soil environment in a growing extent, due to the increasing use of Zn-containing mineral and organic fertilisers, sewage sludge and compost in agriculture, beside atmospheric deposition of Zn-loaded particulates originating from power plants, extractive industries and other types of combustion activities.

In an experimental study, Bergkvist (2002) tried to develop a model to calculate *changes in the mobility* of trace metals in a sludge treated soil (with Cd as an example), in relation to various soil characteristics thoughout a 100 years perspective. Parameterization of the model was derived from a long-term plant nutrion experiment performed at Ultuna, Sweden. Mass balances revealed that Cd had a very low mobility in the sludge treated soil, with about 100 % of the supplied metal retained in the upper 60 cm, most probably due to binding to $CaCO_3$ and pH values between 6.5-7.5. Established adsorption isotherms and k_d-values increasing from 22 l/kg in the sludge-treated mother soil (natural pH of 5.14) up to 163 l/kg in the soil layer below the mother soil, confirmed that pH was the most important soil factor in controlling Cd solubility. By calculating the retarded Cd fluxes in the soil solution based on the established k_d-values, the author assumes a migration velocity for Cd with the soil porewater from the upper layer down to the drainage base of about 4700 years, if macropore flow and colloidal transport are not considered. Soil Cd profiles suggest that Cd transport in the porewater may happen along soil micropores, where sorption on solid phases can take place. It is stated that the model could well describe how mobility and plant-uptake of Cd are controlled by the actual Cd input, pH changes, soil physical characteristics, degradation of organic materials, like humic substances, sludge, green plant residues, or by transport within the plant.

Antoniadis and Alloway (2001) studied the availability of Zn, Ni and Cd to ryegrass (*Lolium perenne*) in sewage-sludge treated soils as a function of temperature (15 and 25 °C) and application rate (0, 10, 50 t/ha) by means of *pot experiments* and *soil ($CaCl_2$) extractions* during a 2-years period. Concentrations of **Ni** and Cd in ryegrass and soil extracts increased as expected after 1 year, but reached a plateau in the second year. In contrast **Zn** in ryegrass did not increase during the first year but as $CaCl_2$-extractable form in the soil. Also in the first year, a rapid decrease of the organic matter was observed, which slowed down in the following year. *Temperature* had a signficant effect in that both plant and soil-extractable metals showed higher concentrations at 25 °C than at 15 °C most probably due to increasing organic decomposition rates at higher temperatures. Also *soil-plant transfer coefficients (Tc)* were higher at 25 °C than at 15 °C and decreased in the order Cd > Zn > Ni.

The authors further demonstrated that also elevated application rates caused greater Tc values for the metals studied indicating that the *sludge-borne metals* added were less strongly bound to soil surfaces than the indigenous metals before sludge addition (see section 5.5.6 above). They conclude that extractability and availability of metals is increasing in soils even in sludge applications similar to rates applied in common agricultural practice (8-10 t/ha) and especially under warmer conditions. The decrease of metals in ryegrass over time at 15 °C may be explained by the weaker growth of the plants, compared with plants grown at 25 °C. On the other hand, that Zn in ryegrass did not increase over time points out to the fact that this plant may have developed a physiological adaptive mechanism to maintain internal concentrations of this essential element over time at optimal levels. In contrast, the increase of plant and extractable Cd and Ni over time happened concurently with the decomposition of the organic matter in the soil-sludge mixture. Other works (references given in Antoniadis and Alloway 2001) also provide evidence that *metal extractability* increases even over a longer (nine years) period due to organic matter decomposition. The *organic degradation* leads to the breaking of humic macro-molecules and the formation of more labile metal forms and soluble organo-metallic complexes with low molecular weight organic ligands (DOC), which are easier for the plants to absorb (from soil solution or loosely bound onto soil surfaces).

Ciba et al. (1997) performed *composting experiments* to follow up the change of chemical forms of **Zn** added as metallic Zn and ZnS to a municipal solid waste material during the composting process under oxygen conditions (exothermic process) by means of sequential extraction (according to Rudd 1988, cited in Ciba et al. 1997). The authors conclude from their composting experiments that composting is increasing Zn mobility mainly due to an increase of Zn associated with carbonates. In general, Zn added as metallic Zn dust remained in mobile and bioavailable forms (organic and carbonate), while Zn added as ZnS remained mainly in a not easily plant-bioavailable form. Concerning composting temperature, the addition of Zn to the compost decreased maximum temperature probably due to toxic effects caused by Zn to soil microbiology.

Richards et al. (2000) examined the *effect of sludge processing*, soil type and pH on metal mobility by using undisturbed soil columns. As expected from what has been said before, they found that soils with composted sludge (with less organic material) and sludge ash showed the lowest overall metal mobility. In a study to estimate the potential environmental impact of metals in sewage sludge from different waste water treatment steps, Alvarez et al. (2002) used the BCR sequential extraction scheme. One main result of their study was a clear rise of more labile metal fractions in the sludge, along the sludge treatment chain. Also Oudeh et al. (2002) tried to find out, by means of pot experiments and chemical extraction, whether the organic content and mycorrhizal status of sludge-treated soils affected the plant and microbial exposure to Cd, **Cu** and **Zn**. Here, extractable metals were higher and responses to inputs more pronounced in the arable, lower organic matter soil, except for Cd in the $CaCl_2$ fraction. Uptake of each metal was higher in the larger plants of the higher organic matter, grassland soil. With the exception of Cd (roots) and Zn (shoots), higher inputs of sludge metals did not increase plant metals. Cornu et al. (2001) observed an increased transport of metals (and DOC) in the dissolved form following sludge application on soil. In particular **nickel** turned out to be most mobile in sludge and treated soil. But no significant increase in total soil metal contents due to sludge spreading was noticed.

In contrast, total metal concentrations increased about 2.7 fold in the final compost of a swine manure due to organic matter decomposition, whereas metal distribution in the different chemical fractions proved to be rather independent of composting age, and hence of the total metal concentration. However, changes in water-soluble **Cu**, Mn and **Zn** fractions were well correlated to the variations of the water-soluble organic carbon concentration (Hsu and Lo 2001).

In a comprehensive literature review, Ekvall (1995) identified a variety of still unresolved difficulties when relating metal speciation in sewage sludge or soils based on single chemical extraction to plant uptake and toxicity. Own experiments showed that Cd and **Zn** in sludge were extractable by weak extractants, such as NH_4-acetate and Na-acetate, but that none of these extractants could remove more than 36 % of total Pb. Most **Cu** was bound to the organic sludge fraction. EDTA was identified as the extractant that could best remove the toxic fraction, as verified by Microtox testing of the sample before and after extraction. The toxicity measured after adding metals (Cd, Cu, Pb and Zn) to a sludge sample was higher than supposed from the metal concentration present in the electrochemically available and colloidal metal phase. However, alteration of the original sludge metal species composition due to the used incubation solution (of the Microtox test), questions the use of the test method, why alternative solutions were tested, and $NaClO_4$ found more suitable to assess metal toxicity in the sludge. Also Cu toxicity was tested in relation to selected organic substances commonly found in sludge, in addition to model compounds with known Cu effects. Most of these substances decreased Cu toxicity, although some (e. g. nonylphenol, myristic acid or palmitic acid) increased Cu toxicity. Also possible *inter-metallic interactions* were tested. An antagonistic interaction was found between Zn and Cu, although *synergistic effects* dominate and Cu toxicity was higher than would have been expected if only ionic Cu would have been present.

It has been argued by Johansson (2000) that plants may actually act as an effective *"barrier"* against high metal contents (except for Cd) associated with sewage sludge treated soils and consequently may not reflect soil metal concentrations. Indeed, increasing metal concentrations can cause mortality before transfer to the next trophic level may occur (*"soil-plant barrier"*). But we know, metals can be toxic to soil microorganisms (nitrification inhibition!), and so decrease soil quality and fertility. Although total soil metal concentrations may be considered appropriate as a first estimate of the total metal pool available for long-term changes, we have to know the form a metal is introduced with the sludge into soils and how it will change according to prevailing soil characteristics, when assessing its bioavailaibility. By using sewage sludge with varying metal loads added to soils, Johansson (2000) could document that beneficial effects to soil microorganisms occur mostly due to co-*existing soil ameliorating substances* in the sludge, which improve soil structure, and increase the water, phosphorus and positive ion binding capacity of the treated soil. The author states further that rather high amounts of metals will be required to detect possible disturbances of the soil microbial system.

As shown before, the high mobilization of **chromium** observed when Cr-contaminated materials containing significant amounts of organic matter (e. g. compost, sewage sludge, organic-rich soils or sediments) is leached at pH > 8, may be due to its association with DOC. When pH increases DOC, mobilization may also increase the mobility of Cr(III). To provide more detailed information about the buffering capacity of a specific matrix (e. g. soil or sewage sludge) within specific pH domains or intervals, and to get valuable indications about the corresponding sensitivity of these materials to external pH change and stress, the *pH dependence test* has been recently developed by the CEN TC 292 working group, to establish acid/base consumption data required to reach a certain final test pH. Moreover, combining leachability as a function of pH, and of the amount of acid or base consumed to reach a certain pH level, offers the possibility to address also long-term changes in soil material properties and in exposure to external influences (e. g. by sewage sludge application) (see EC-Newsletter "Network on Harmonization of Leaching/Extraction Tests").

5.6 In biota

In many cases, no straightforward relationship can be shown to exist between metal concentration in the tissues of a biological species and metal concentration in the soil or sediment where the organism lives. Exposure of soil- and sediment-dwelling organisms to metals can occur via (pore-) water, soil or sediment particulates, and/or via food. While uptake from and depuration to the water phase are rather well predictable, predictions of transfer rates of metals from soils and sediments to biota are less accurate.

According to the BLM-model (see chapter 6), metal accumulation in fish gills can now be described also as metal desorption from particles, as a result of mucus competition and pH increase. The subsequent increase of available free metal ions will cause the observed final metal accumulation in the gills.

Recent studies have demonstrated that natural organic colloids (1 nm – 0.2 μm) are abundant in seawater and that a significant but variable fraction traditionally defined as dissolved metals actually is in the colloidal form. However, it is still unclear whether complexation of metals with colloidal organic matter enhance or reduce metal bioavailability. Most probably, this will vary depending on the feeding behaviour and other characteristics of the organism considered. Colloid-bound metals (like Zn and Cd) may dissociate from colloidal organic matter and become complexed by transport ligands before transfer through cell membranes. Colloid-bound metals may also be transported by lipid permeation. Given the importance of recent findings on the role of colloids in metal complexation, new models are urgently required, which consider also the transport of colloidal metals across biological membranes.

Exposure of soil and sediment organisms to metals can occur via (pore) water, soil or sediment particulates and the food. While uptake from and depuration to the water phase is rather well predictable, predictions of transfer rates of contaminants from soils and sediments to biota are still less accurate. For all *cationic metal species*, there are two main mechanisms observed as driving forces for metal uptake in plants across the plasma membrane, i. e. the large negative electrochemical potential produced as a result of the membrane H^+ translocating adenosine triphosphate (ATPase), and the transport of metal-chelate complexes. Indeed, new studies suggest that *uncharged metal complexes* (like $CdSO_4^o$) may exhibit *lipophilicity* and may be equally available to aquatic and terrestrial plants as the free Me^{2+} ion. There is also increasing evidence that not only organically complexed metals, but also complexes with inorganic ligands such as chloride may be taken up by plants (for more details see McLaughlin 2002). The following examples have been selected to shortly illustrate the current state of discussion regarding interactions between biota and the bioavailability of metals (see the following chapters 6 and 7 for more details).

Ruby et al. (1999) gave a broad review of in-vivo studies and of the present state-of-the-art on bioavailability-predictive extraction tests. The authors state that current risk assessments of metals in soils are still based on the estimated oral toxicity, but do not take into account characteristics of metals in soils and corresponding limitations regarding their true enteric absorption (see also section 5.4.8.2). The authors stress that a more accurate risk assessment is necessary based on the metal bioavailability found in site-specific soils, relative to the bioavailability of metals administered in toxicity in-vivo studies (*relative bioavailability*), which are however too expensive and time consuming. They suggest a more promising alternative approach by using *in-vitro extraction tests*, which are supposed to be predictive of the *oral bioavailability* of metals from soils. These test procedures may be a more inexpensive and rapid alternative for more accurate exposure estimates, which is required in human health risk estimates. However, to account for the limited dissolution of metals from soil during passage through the gastrointestinal tract, and to derive more accurate exposure rates, it may be still necessary to assess the bioavailability of a metal in soils relative to the bioavailability of metals dissolved in water.

In general, in *vitro extraction tests* include metal salts or metal-containing salts incubated in a low pH solution for a period that mimic residence time in the stomach. Then pH is increased to near neutral values and the incubation continued to mimic residence times in the small intestine, with enzymes and organic acids added to simulate the effect of gastric and small intestinal fluids. The finally dissolved metal fraction is supposed to represent the so-called *bioaccessible fraction*, which is soluble and available for bio-absorption (see also 'European Standard for Safety of Toys': Bioaccessibility of 8 metals in use since 1994 by the 18 members of the CEN to regulate the safety of toys; ref. given in Ruby et al. 1999).

Mineral phases like sulfates that form more under acidic conditions tend to be more stable under the acidic condition of the stomache and so less bioaccessible, in contrast to minerals formed under more alkaline conditions, like carbonates or oxides, which are in turn more bioaccessible in the stomach environment. Oral metal bioavailability is determined by dissolution rate processes, because the metal must first dissolve to become bioavailable. As a rule, it is supposed that less soluble minerals in general dissolve by *surface reaction-controlled kinetics*, which in term are limited by the surface detachment of ions. On the other hand, more soluble minerals dissolve by *transport-controlled kinetics* where dissolution ions are rapidly detached and accumulate to form a saturation solution adjacent to the mineral surface. Consequently, the dissolution rate is controlled by the rate of ion transport away from this saturated layer.

To summarize, the review by Ruby et al. (1999) revealed a good correlation between in vitro extraction tests and in vivo data (e. g. from animal tests etc.) as a *relative bioavailability estimate* of metals. Perhaps noteworthy in this context for the reader that a 'Solubility/Bioavailability Research Consortium' (SBRC) stakeholder group was formed in 1997 to promote the acceptance of and validate in vitro methods to estimate metal bioavailability from soils.

In general, there is not a straightforward relation existing between metal concentrations in biological species and in soil or sediment, although Rai et al. (2002) very recently demonstrated that this can occur. They found a positive correlation between metals (Pb > **Cr > Cu** > Cd) in the edible aquatic macrophyte (crop) *Euryale ferox Salisb.*, and metal contents in both the water and sediment. Also, in a field survey on aquatic macrophytes taken from metal-contaminated urban streams in Queensland/Australia, plant roots analyzed exhibited a higher metal load (Cd, Cu, Zn, Pb) than the ambient sediment. However, a significant pattern of increasing plant accumulation with increasing sediment concentration was only observed for **Zn** (Cardwell et al. 2002).

Laing et al. (2002) studied the bioavailability of metals to the spider *Pirata piraticus* in the intertidal sediment zone of the Scheldt estuary and observed higher metal contents (Cd, **Cu, Zn**) in spiders at lower total sediment metal concentrations. They found a strong positive correlation between Cl^- in the sediment and the metal content in spiders, as well as a strong relationship between exchangeable **Zn** and the Cd/CEC ratio, and the metal contents in *P. piraticus*. The authors resume that salinity, CEC and exchangeable metals are the most important factors for metal bioavailability in this sediment.

When comparing three trace metal contaminated sites to evaluate possible changes of the existing indigenous biota, Murray et al. (2000) could show that corresponding plant metal levels (Cd, **Cu, Ni**, Pb, **Zn**) were rather influenced by *soil characteristics* and not by total metal concentrations in the soil.

Also contrary to previous assumptions, Gray et al. (2001) found that *periphyton metal accumulation* studies could be misleading to indicate ambient water levels, when conducted in systems where *ferromanganese oxide concentrations* are elevated, due to the metal-sorbing potential of Fe and Mn oxides coexisting within the periphyton matrix. Similarly studies conducted by Behra et al. (2002) confirmed that the metal content in periphyton may not be a straightforward measure of metal contamination and bioavailability. Their results proved that non-exchangeable (intracellular and so not adsorbed) **Cu and Zn** concentrations in periphyton may occur at least in one river, where periphyton metal concentrations did not reflect dissolved metal contents (see section 5.2.1).

Klerks and Fraleigh (1997) conducted shortterm uptake experiments with **Ni and Zn** radioisotopes and the zebra mussel *Dreissena polymorpha.* Despite a low affinity of Ni for particulates, the uptake of particulate Ni was significant although less important than the uptake of dissolved Ni. The uptake of Zn by the mussel varied from only dissolved Zn uptake to only particulate Zn uptake depending on the composition of the suspended particulate. Also, Zn uptake was higher in high turbidity water. Yet, half of the accumulated Zn was excreted again after 24 h, while Ni was not excreted at all. So most of the Zn was biodeposited as feces, while most of the Ni remained bioaccumulated. One conclusion drawn from this study was that tissue levels used in biomonitoring programs may reflect both *dissolved and particulate metal levels*, but also the capacity of organisms to *adapt* to certain trace metal levels (see section 7.3).

MacFarlane and Booth (2001) admit that natural differences in the physicochemical composition of the sediment may be main determinants for the prevailing *macrobenthic species assemblages* in estuarine creeks, where metal levels have been found below existing sediment quality guidelines established to predict biological effects. But biotic assemblage patterns are not maintained temporally, why monitoring over time becomes necessary. Their study strongly emphasizes the obvious difficulty in relating macrobenthic responses to low-level contamination at the community level.

Tao et al. (2002) exposed fish (carp) to **Cu** adsorbed to kaolin particles at various concentration levels. They determined Cu adsorption affinity constants with varying pH and used the obtained experimental data to quantitatively model the relationship between affinity constants and pH by a speciation code (MINTEQA2). Their modelling results showed that the observed *Cu accumulation in fish gills* was due to a desorption of this metal from kaolin particles, as a consequence of mucus competition and a slight pH increase. The increase of available Cu was supposed to finally cause the observed Cu accumulation by the gills (cf. chapter 6).

Recent studies demonstrate that *natural organic colloids* (1 nm – 0.2 µm) are abundant in seawaters and that a significant but variable fraction of traditionally defined dissolved metals is in the colloidal form. However, it is still unclear whether *complexation of metals with colloidal organic matter* enhance or reduce metal bioavailability. In previous studies, the dissolved metal phase was defined as the fraction passing through a 0.45 µm membrane and thus included also colloidal metals. Wang and Guo (2000) examined the bioavailability of colloidally complexed Cd, **Cr** and **Zn** to two marine bivalves (green mussel *Perna viridis* and clam *Ruditapes philippinarium*) by means of radiotracer and ultrafiltration techniques to measure metal uptake from the colloid phase. Their experiments showed that the influence of metal complexation with colloidal organic matter varied among metals but was highly ligand species specific. Cr considered as a transitional metal prefers to bind in general to *O-containing ligands*, while Zn (and Cd) as transitional type B metal prefers to bind to *S-containing* rather than *N- and O-containing ligands*. Zn bioavailability was almost completely inhibited by its complexation with high molecular weight dissolved organic matter (HMW) compounds, in agreement with recent studies, in which colloidal complexation reduced Zn bioavailability to both phyto- and zooplankton.

In contrast, Cr(III) showed an increase of the bioavailability to mussels when associated with HMW colloids, although a further increase of organic colloids reduced again its uptake. As most Cr was in the form of colloidal complexes, the higher uptake may be due to an increased sorption onto the bivalve's tissue or direct uptake through pumping of colloidal particles (ingestion).

However, total uptake (on a dry weight basis) was highest for Zn (and Cd) maybe due to different binding affinities of these metals with SH-containing compounds, e. g. protein ligands (presumably membrane transport proteins) in the marine bivalves. The authors hypothize that colloid-bound metals (like Zn and Cd) may dissociate from colloidal organic matter and become complexed by *transport ligands* before internalization. They further speculate that due to the *amphiphillic nature of colloidal organic matter*, colloid-bound metals may also be transported by *lipid permeation*. About > 60 % of Zn was found in the mussels' soft tissue, and about 20 and 10 % in the shell and digestive gland, respectively, independent of the colloidal organic carbon content. In contrast, Cr was almost evenly distributed among the shell, digestive gland and the remaining soft tissue. A much higher percentage of Cr, Zn (and Cd) was associated with the shell of the clam. Only about 20 % of Cr (and Cd) were in the remaining soft tissue and digestive gland. The authors concede that it was difficult to maintain all the radiolabeled metals in the colloidal form during the exposure period and that re-partitioning/desorption of radiolabeled metals from the colloidal phase to the truly dissolved phase can not be avoided.

It is summarized that colloidal complexation may *reduce bioavailability* of certain metals (e. g. Zn) due to a *decrease in free or inorganic metal concentrations*. In turn it may *increase bioavailability* for some metals (like Cr) due to *surface sorption or ingestion* by filter feeding animals. Given the importance of recent findings on the occurrence of colloids in metal complexation, new models are urgently required, which consider also the transport of colloidal metals across biological membranes.

There is sufficient evidence that significant accumulation differences exist between different taxonomic groups. For this reason, risk assessment methods need more information on *feeding behaviour* of exposed organisms. Heikens et al. (2001) calculated regression equations based on total soil and body concentrations for various metals and various taxonomic groups of invertebrates. They found that the *internal metal load* increased with soil metal concentration for most taxonomic groups in the order: Pb > Cd > **Cu**, in contrast to the **Zn** body load, which remained rather constant over the observed range of soil metal concentrations, indicating an *adaptive uptake mechanism* of the exposed animals.

The use of *microbial biosensors* has been increasingly proposed as a practical alternative to chemical speciation to assess metal bioavailabilities, also due to lower operational costs (Flynn et al. 2002). Madoni (2000) reported a freshwater ciliate, *Spirotomum teres*, which may serve as an excellent bioindicator for toxic metals, in particular for **nickel**. In contrast, Gundacker (2000) reported that the high environmental metal load found of Danube harbours was poorly reflected by sediment bioindicator species. Also ‘gastropods’ showed a 20-fold higher **Cu** concentration than bivalves, suggesting a higher Cu regulation capacity of the latter. Due to their closer sediment association, bivalves seem to be more appropriate to reflect Cu concentrations in fine-grained sediment fractions. A review of studies using bivalves as sentinel bioindicators in marine sytems impacted by trace metals was given recently by Boening (1999). Tibazarwar et al. (2001) report the use of a bacterial strain (*Ralstonia eutropha* AE2515) as highly selective for **Ni** (and Co) in soil, and suggest its use as biosensor to quantify Ni (and Co) bioavailability, accumulation in agricultural crops and transfer (of Ni and Co) to higher trophic levels.

5.7 A proposal for “natural” or “preindustrial” regional background levels of metals in the sediment in waters surrounding Stockholm

In order to classify the quality of aquatic sediments, a set of “natural” or “comparative values” is required, which can be expected for systems without any human impact. However, it is a well established fact that these “natural”, rather geologically derived background values are not identical for an entire country, due to variations in bedrock geology. For this reason, the “natural background” concentration of metals must also differ from region to region. In this context it is difficult to determine regional background concentrations for metals in areas that have been exposed to contaminants released from human activities over long periods of time. Moreover, natural processes, such as bio-turbation, sediment resuspension and elevation of deposition bottoms, may overlap and confuse the build-up of stable sediments. Thus, sampling and analysis of the metal content at a certain sediment depth might not automatically provide unbiased data on “pre-historical” or natural levels of metals.

This dilemma can be resolved by looking for metals in the sediment, which exhibit very low concentrations at a certain sediment depth, and which are known at the same time to be typical anthropogenic contaminants, but untypical for the geological formation of the study area. This would strongly indicate that the sediment layer in consideration is uninfluenced by human activities. Consequently, the concentrations of other trace elements could also be interpreted as "natural background levels". Examples of such indicator elements, to be used for selecting sediment samples with natural background concentrations of other trace metals, could be mercury and cadmium.

The Swedish SQCs for both freshwater and marine sediments establish, as a basis for sediment quality classification, a set of "natural" or "comparative values" that are to be expected if there was no human impact. These "natural" or "original" metal concentrations are set as uniform levels for the whole country (and its coastal waters). However, it is a well established fact that these rather geologically derived background values are not identical for the entire country, due to the varying bedrock geology. For this reason, the "natural background" concentration of the different trace metals must also vary from region to region.

It is sometimes argued that it is very difficult to determine the regional background concentration of trace metals in sediment, at least in areas that have been exposed to contaminants released from human activities over very long periods of time. Moreover, a number of natural phenomena, such as bioturbation, sediment resuspension and elevation of deposition bottoms, may confuse the regular build-up of stable sediments (compare section 5.4.6). Thus, sampling and analysis of the metal content at a certain sediment depth might not automatically provide unbiased data on "pre-historical" or natural levels of metals.

A possible way of resolving this dilemma may come from the fact that there are metals not necessarily related to a specific geological formation. Therefore, if certain trace elements, known to be typical anthropogenic contaminants and, at the same time untypical for the geological formation in the study area, exhibit very low concentrations at a certain sediment depth, this may be a strong indication that the sediment layer in consideration is uninfluenced by human activities, and that the concentrations of other trace elements at the same time could be interpreted as "natural background levels". Typical candidates of such indicator elements, to be used for selecting sediment samples with natural background concentrations of other trace metals, could be mercury and cadmium.

This approach has been tried on a material presented by Östlund et al. (1998), covering trace metal data, obtained by means of digestion and analysis according to Swedish Standard, from altogether 394 samples taken from 117 sediment cores collected in the close surroundings of the city of Stockholm, both from Lake Mälaren, the inner archipelago (Baltic Sea) and from some smaller lakes within the borders of Greater Stockholm. The criteria for selection of samples from the database were:

- ✓ the deepest layer analysed in each specific sediment core (usually at sediment depths of more than 40 cm, but occasionally at around 30 cm depth);
- ✓ LOI (loss on ignition) in general >7% (mostly in the range 7-10%, in a few small lakes up to 49%);
- ✓ total concentration of Cd <0.4 μg/g DS and total concentration of Hg<0.1 μg/g DS.

The justification for choosing these cut-off values for Cd and Hg was a previous set of nation-wide trace metal background concentrations in lake sediments, proposed by SEPA in 1991.

Altogether 15 sediment samples fulfilled the above criteria, and these were examined with respect to the concentrations of a certain number of trace metals, (see Table 5.16).

Table 5.16. Possible regional background concentrations of some trace metals in uncontaminated sediment layers from 15 sediment cores, collected in the Stockholm area. Concentrations are based on digestion of samples in 7 N nitric acid (Swedish Standard) and are expressed as μg/g dw (from Landner, 2002).

Trace metal	Conc. range	Corrected* range	Arithmetr. Mean	Median
Cadmium	0.10 – 0.35	---	0.22	0.20
Mercury	0.009-0.074	---	0.033	0.036
Copper	34 – 47	34 – 42	38	37
Chromium	40 – 61	40 – 58	52	52.5
Nickel	27 – 40	27 – 37	33	33
Lead	17 – 51	17 – 31	21	20.5
Zinc	100 – 180	100 – 160	122	120

* Correction means that one single outlier has been omitted. Mean values are computed from the corrected range.

As can be seen in Table 5.16, the metal concentration ranges in the 15 samples are quite narrow, and in most cases, the mean or median values are close to the minima. This makes it possible to draw the conclusion that it is likely that the real regional background level of Cu in the Stockholm area is 37-38 µg/g DS (i.e. not 15 µg/g DS, as indicated in the nation-wide background data). The corresponding background levels for Ni is 33 µg/g DS, for Pb about 20 µg/g DS and for Zn about 120 µg/g DS. Therefore, it seems reasonable to make assessments of the degree of metal contamination of the inner water-ways of Stockholm by comparing measured concentrations with the above proposed "regional background levels", at least until some more reliable background data for the geological domain of eastern Svealand are available.

The above proposed regional background levels for Cu and Zn in Lake Mälaren sediments turn out to be quite similar to very recently established levels for *"preindustrial sediments from the Strängnäs area"* (Strängnäs being a city on Lake Mälaren upstream of Stockholm) by Lithner et al. (2003), of 35 µg Cu/g DM and 130 µg Zn/g DM. These authors also indicate that the Cu and Zn concentrations in *"unaffected superficial sediments upstream Stockholm"* may be estimated at around 50 µg Cu/g DM.

6 BIOTIC LIGAND MODELS

Significant advances have been made in recent years to improve our understanding of the mechanisms by which trace metals exert toxic effects on aquatic life. Moreover, much has been learned about how site-specific differences in water quality can affect metal speciation, and that it is the metal species that affects its bioavailability and toxicity to aquatic life.

It has repeatedly been shown that differences in the free metal ion activity (in the case of copper, Cu^{2+}) of the test medium can explain a substantial part (but not all) of the observed toxicity variability. But also other Cu forms (like complexes with OH and CO_3 ions and organic metabolites) have the potential to contribute to Cu toxicity. It is also assumed that competition between Ca^{2+}, Mg^{2+}, Na^+ and H^+ ions and Cu ions for binding sites at the organism-water interface may result in a decreased toxicity of the free cupric ion. Analogous phenomena have been shown to exist for Zn. Recently developed "Biotic Ligand Models" (BLMs) include all these aspects and are, therefore, gaining increased interest in the scientific as well as the regulatory community. In fact, the BLM concept, now developed for Cu, Ni, silver (Ag) and Zn, is considered as the currently most practical technique to assess the ecotoxicity of metals on a site-specific basis. Therefore, the BLM concept is now being approved in the EU.

A basic assumption of the BLM is that metal toxicity occurs as the result of metal ions reacting with binding sites at the organism-water interface, represented as a metal-biotic ligand (metal-BL) complex. The concentration of this metal-BL complex directly determines the magnitude of the toxic effect, independent of the physical-chemical characteristics of the test medium. Hence, the acute toxicity of a trace metal to an organism can be calculated when metal speciation, the activity of each cation in solution, and the stability constant for each cation to the BL(s) for the organism are known.

BLMs were then rapidly developed for fish (rainbow trout, fathead minnow) and crustaceans to predict acute toxicity of Cu, Ag and Zn. Later on, the BLM approach was shown to be applicable also to the assessment of chronic toxicity to the same organism groups as well as to green algae. It was furthermore demonstrated that concentrations of Cu and Ni in the gills of fathead minnows were constant predictors of acute toxicity of these metals to the fish, although water hardness varied up to 10-fold. In contrast, concentrations of total Cu and Ni in the water or free-ion activities of these metals could not be used as good toxicity predictors.

The "critical BL concentration", i.e. the amount of metal bound to the fish gill that caused 50% mortality at 96 h, was determined as 250 nmol Ni per g wet weight (ww) and, for copper, about 12 nmol/g ww. Other researchers provided only slightly modified values of the "critical BL concentrations" in gills of fathead minnows: for Cu – 10 nmol/g, for Ag – 17 nmol/g and for Ni – 240 nmol/g ww. The corresponding value for Cu in gills of Daphnia magna *was about 12 nmol/g and for Ag – 2.3 nmol/g.*

Later work concentrated on the problem of predicting chronic toxicity on the basis of the BLM. It was shown that the BLMs for rainbow trout, Daphnia magna *and a green alga were able to significantly reduce the variation in effect concentrations, i.e. the chronic toxicity of Cu and Zn to these organisms was generally predicted within a factor of two from the observed values, both when using laboratory waters and waters collected in the field. It was also demonstrated that dietary uptake of Cu in daphnids did not enhance the chronic toxicity of waterborne Cu.*

6.1 Need for improved models to assess bioavailable fraction of metals

In a regulatory context, water quality criteria (WQCs) should be defined on the basis of sound scientific information and ecotoxicological principles as concentrations of a specific chemical in water that will protect aquatic life and human health. WQCs should serve as a basis for developing watershed based control programs. If we take copper toxicity to freshwater organisms as an example, it can be noted that when similar concentrations of copper are compared in side-by-side toxicity tests using laboratory and natural water samples, comparable effects are generally **not** observed. The adverse effects observed in the laboratory systems are typically mitigated, to varying degrees, in most natural water samples. This observation is important, since the WQCs that have been established to protect aquatic life are normally based on the results of such clean water laboratory toxicity tests (DiToro et al., 2000).

Still today, it can be noted that WQCs and environmental risk assessment procedures for trace metals are predominantly based on total or (at best) dissolved metal concentrations. This is at least still the case in many European countries. However, there is currently extensive evidence that neither total nor dissolved aqueous metal concentrations are good predictors of metal bioavailability and toxicity.

Regulators in the United States have been a little more receptive to the new scientific view than their European colleagues in the development of environmental risk assessment schemes for metals in water and in the establishment of WQCs. For example, the U.S.EPA allows site-specific adjustments to the WQCs, such as the determination of water effect ratios (WERs).

The first consideration of site-specific differences in establishing WQCs was made in relation to water hardness. In fact, the WQC for metals such as cadmium, **copper**, lead**, nickel** and **zinc** are formulated in a way that accounts for the observation that the toxicity of these metals is inversely related to water hardness. Thus, as hardness (Ca + Mg) increases, it takes a higher metal concentration to achieve the same effect, and as a result, the LC50 increases.

This dependence of the WQC on water hardness was clearly shown in the original American freshwater copper WQC document (U.S.EPA, 1985). However, it was also recognized in the original document that water quality characteristics other than hardness, including both pH and DOC, will also affect the toxicity of copper. Since the precise reasons for this were not well understood at the time, and available data were not sufficient, these aspects were not incorporated into the criteria document of 1985. As it turns out, these other effects can be substantial, so that failure to consider them can lead to an inefficient use of resources to control the discharge of copper, when in fact it may not be warranted (DiToro et al., 2000). It is for this reason that the U.S.EPA has recently recognized the need for updated evaluation procedures (Renner, 1997).

In a relatively recent paper, it was even demonstrated that natural organic matter (NOM) of different origin may have quite different capacity of reducing the accumulation and the toxicity of metals in fish (Richards et al., 2001). In general, NOM is a complex mixture of organic compounds including humic and fulvic acids as well as many types of carbohydrates, proteins and lipids. NOM can be produced within the water column, e.g. from phytoplankton (autochthonous NOM) or washed in from the surrounding catchment (allochthonous NOM). Richards et al. (2001) showed that increasing concentrations of NOM from different sources increased survival of rainbow trout exposed to a mixture of dissolved trace metals (Ag, Cd, Co, **Cu**, Hg and Pb), but the NOM having the most allochthonous properties increased fish survival most, while the NOM with the most autochthonous properties increased it the least.

In spite of a long history of sceptical attitudes, the concept of bioavailability has recently gained increasing recognition by the regulatory side also in European countries. Although current WQCs for metals do neither cope with new knowledge on metal speciation, nor consider the question of how metals exert toxicity, they have now, for the first time, started to include at least some aspects of *bioavailability*. For example, they nowadays sometimes refer to *dissolved metal concentration* as a supplement to *total recoverable metal concentration*, or there may be an adjustment for some main parameters, like water hardness. But there are many more factors in natural waters known to influence metal bioavailability. It is, for example, today well established that the toxicity of copper to freshwater fish depends on a variety of factors, like pH, DOC and alkalinity, in addition to hardness.

Thus, significant advances have been made in recent years with respect to understanding the mechanisms by which trace metals, such as copper, exert toxic effects on aquatic life. In addition, much has been learned about how site-specific differences in water quality can affect metal speciation, and that it is the *metal species* that affects its bioavailability and toxicity to aquatic life.

A large number of recent studies have shown that differences in the free metal ion (Cu^{2+}) activity of the test medium can explain a substantial part (but not all) of the observed toxicity variability. This supports the "Free Ion Activity Model" of toxicity, the FIAM model, formulated by Morel (1983) and critically reviewed by Campbell (1995). But we also know that other Cu forms, like hydroxy complexes (Allen and Hansen, 1996) or complexes with low molecular weight metabolites, have the potential to contribute to the observed Cu toxicity.

Another important aspect when assessing how copper toxicity is determined was pointed out by Playle et al. (1993), DiToro et al. (2001), Santore et al. (2001) and De Schamphelaere and Janssen (2002). This is the assumed competition between Ca^{2+}, Mg^{2+}, Na^{+} and H^{+} ions on one hand and copper ions, on the other, for binding sites at the organism-water interface (e.g. fish gills). The presence of these ions consequently results in a decreased toxicity of the free copper ion.

In his PhD thesis, De Schamphelaere (2003) gives a brief survey of the mechanisms of copper toxicity in fish and invertebrates and points out that whereas the mechanisms of acute toxicity are well understood, chronic toxicity may be the result of a complex combination of different mechanisms. The gill is a multi-functional organ serving the purpose of respiration, nitrogenous waste excretion, acid-base balance and osmoregulation. The key target for acute copper toxicity in freshwater fish appears to be sodium homeostasis, although chloride absorption and nitrogenous waste excretion can also be influenced (Grosell et al., 2002). The net loss of sodium (and chloride) creates an osmotic imbalance between plasma and tissues. Via a complex cascade of events, this eventually leads to cardiovascular collapse, resulting in death. Mortality occurs when plasma sodium concentrations are reduced by about 30% (Wilson and Taylor, 1993).

A similar mechanism is expected for invertebrates, although these may have different tolerance for reduced heamolymph concentrations (Grosell et al., 2002).

Also in the case of zinc toxicity in the aquatic environment, it has been known for quite some time that this is modified by a number of abiotic parameters such as pH, hardness and dissolved organic matter. The modification of zinc toxicity by water hardness was taken into account in the WQC document for zinc in 1987 (U.S.EPA, 1987). However, already in 1980, the reported antagonistic effects of Ca^{2+} and Mg^{2+} on metal toxicity was attributed to increased competition for binding sites on sensitive tissues or a lowering of the ionic activity of the metal (U.S.EPA, 1980). A great number of authors have demonstrated the effect of pH on zinc toxicity, but to some extent, their conclusions are contradictory, since some authors have reported the highest toxicity to fish and crustaceans at the highest pH, while others observed a reduced zinc toxicity to crustaceans at increasing pH (Heijerick et al., 2002a).

From the above cited observations, it is clear that next to trace metal speciation, which determines the free metal ion activity, interactions at the site of toxic action also need to be considered when evaluating bioavailability of metals such as copper, nickel and zinc (Meyer et al., 1999; DiToro et al., 2001; De Schamphelaere and Janssen, 2002). Recently developed "Biotic Ligand Models" (BLMs) include both aspects and therefore, they are gaining increased interest in the scientific and regulatory community (Playle, 1998).

In fact, the BLM concept, now developed for copper, nickel, silver and zinc, is considered as the currently most developed, practical technique to assess the ecotoxicity of metals on a site-specific basis, i.e. taking into account differences in the physico-chemical characteristics of the surface water. This is obviously one important reason for the recent approval of the BLM concept by the European Union and its Member States at their Technical Meeting on the Evaluation of Existing Substances in early June 2003 in Italy. It is felt that the acceptance of the BLM concept is of great relevance for the assessment of many trace metals currently undergoing official (**Ni**, Sb and **Zn**) or voluntary (**Cu** and Pb) substance risk assessments at the EU level, and also critical for a fair evaluation of trace metals in the New Chemicals Policy (Eurometaux, 2003).

6.2 Development of Biotic Ligand Models

A great number of papers by Playle and co-workers over the years 1992-2000 (see e.g. Playle et al., 1992; Playle, 1998; and McGeer et al., 2000), showed that uptake of various cationic metals, including cadmium, cobalt, **copper** and silver, onto fish gills leads to organism mortality, and that the uptake of the metals could be described by conventional chemical equilibrium models, in which the gill was treated as a ligand described by a stability constant and reaction site concentration. In their work, they excised fish gills and analysed the concentration of metals on the gill surface. The toxicity of dissolved metals was shown to be proportional to the accumulation of the metal on the gill (Allen, 2002). Thus, a considerable amount of thermodynamic information on metal accumulation at the biotic ligand (BL) is based on the studies of Playle and co-workers (e.g. Playle et al., 1993). Site concentrations and binding constants, e.g. for interactions between fish gill and metals, hydrogen, and cations, provide a functional description of the accumulation, while critical BL concentrations are based upon dose-response relationships between mortality and accumulation at the gill.

A basic assumption of the BLM is that metal toxicity occurs as the result of metal ions reacting with binding sites at the organism-water interface (either physiologically active sites, leading to a direct biological response, or transport sites, leading to metal transport into the cell followed by a subsequent, indirect biological response), which is represented as the formation of a metal-biotic ligand (metal-BL) complex. The concentration of this metal-BL complex directly determines the magnitude of the toxic effect, independent of the physical-chemical water characteristics of the test medium (De Schamphelaere and Janssen, 2002). Hence, the acute toxicity of a trace metal (percent response) to an organism can be calculated when metal speciation, the activity of each cation in solution, and the stability constant for each cation to the BL(s) for that species are known (Heijerick et al., 2002a).

The BLM mathematically integrates the interaction of trace metal with solution phase ligands to predict its speciation and its subsequent interaction with receptor sites on the organism. It is, thus, obvious that the BLM includes both the chemical and the biological parts, which enable it to predict toxicity of dissolved metals based on the accumulation of the metal on the receptor site (the gill or other biological membrane in contact with the surrounding medium) (cf. Allen, 2002).

The BLM can be used as a computational alternative to bioassays, for example, to evaluate site-specific WQCs. Describing bioavailability as a function of speciation has so far been based on the free ion activity model (FIAM). Following the basic concept of the gill surface interaction model (GSIM), developed by Pagenkopf (1983), the BLM has further developed this approach by including competitive interactions at the site, where acute metal toxicity occurs. Thus, BLM is based on the hypothesis that that toxicity is not simply related to the total or dissolved metal concentration, but is dependent on the metal speciation in the water column and interaction processes at the site of toxic action (Figure 6.1). By simulating metal accumulation at this particular site (defined as the BL), toxicity is defined according to the BLM as the metal accumulation beyond a critical threshold. For fish, for example, the BL is either known or suspected to be the sodium or calcium channel proteins in the gill surface that regulate the ionic composition of the blood.

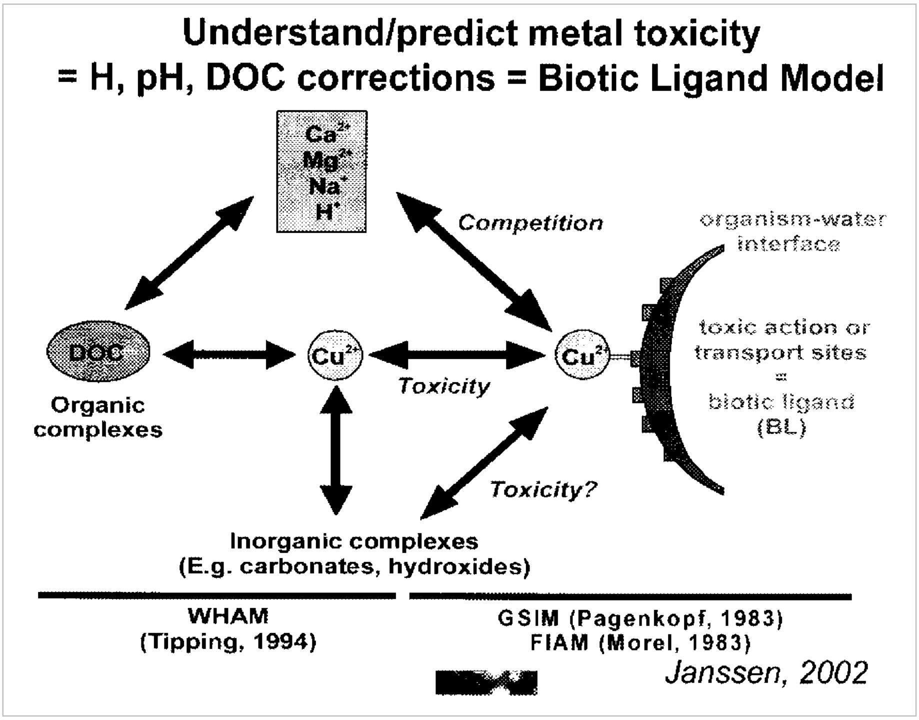

Figure 6.1. Conceptual diagram of the Biotic Ligand Model (BLM) showing inorganic and organic complexation in the water and interaction of metals and cations on the biotic ligand (BL). DOC = dissolved organic carbon. Modified after Pagenkopf's (1983) original GSIM model.

For other organisms, it is hypothesized that a BL exists and that mortality can be modelled in a similar way. The BL interacts with the metal cations in solution. The amount of metal that binds is determined by a competition for metal ions between the BL and the other aqueous ligands, particularly NOM, and the competition for the BL between the toxic metal ion and the other metal cations in solution, for example, calcium (DiToro et al., 2001).

In the BLM, the cation-BL interactions are represented and calculated in the same way as any other reaction of a cation with an organic or inorganic ligand. To that end, the complexation capacity of the BL (analogous to the concentration of other ligands in solution) and stability constants for the metal- and the cation-BL complexes are required and need to be incorporated in a speciation model like the "WHAM" (De Schamphelaere and Janssen, 2002). The "WHAM" or "Windermere Humic-Aqueous Model", developed by Tipping (1994), predicts the interaction between metals and organic matter, based on a relatively simple characterization of the sample for its description. The model has been calibrated with a large number of samples of NOM from a wide variety of locations.

The first BLMs predicting acute copper toxicity to fish combined existing data sets on both gill-copper concentrations and stability constants, as well as on toxicity and incorporated them into the metal speciation model "Chemical Equilibria in Soils and Solutions" (CHESS), which includes speciation reactions, adsorption on natural surfaces and solubility control of solid surfaces (Santore and Driscoll, 1995). Also, electrostatic and chemical interactions between metals and natural organic matter were considered, e.g. the metal-DOC (or metal-NOM) complexation according to WHAM was included.

It is today generally recognized that the BLM approach permits a rational explanation of the effect of variation in water chemistry on the toxicity of metals to aquatic organisms. Presently, bioassay testing often is applied to ascertain if there is a difference in toxicity at a site from that anticipated by the WQC. However, it is much more cost-effective and introduces less uncertainty if the BLM is used to predict toxicity under a widely varying set of water quality conditions. Therefore, as pointed out by Allen (2002), the BLM promises to be an important tool for use in any scenario where that requires an understanding of bioavailability in relation to water chemistry.

To further demonstrate this conclusion, Santore et al. (2001) used a database from Erickson et al. (1996) on copper toxicity to fathead minnow (*Pimephales promelas),* measured in a wide range of different water qualities (pH ranging from 6.5 to 8.8, Ca^{2+} from 0.5 to 2.5 meq/l , and DOC from 1-6 mg/l). He could clearly show that total copper in water never is a good predictor of copper toxicity, that Cu^{2+} only is a constant predictor of copper toxicity when DOC changes, but that gill copper binding is a constant predictor of toxicity as pH, DOC and hardness varies. In fact, the LC_{50} of copper varied between 10 and 1,000 µg/l when copper was expressed as $Cu_{dissolved}$, but only between 5 and 12 nmol/g (wet weight) when copper was expressed as “gill-Cu” concentration. Van Sprang (2003) later demonstrated that a significant reduction in intraspecies variability in copper toxicity can be obtained through incorporation of bioavailability (use of BLM) in a wide variety of fish, crustacean and algal species.

To summarize, it is clear that, initially, the BLM concept was to a large extent developed using data from the scientific literature. In fact, a first BLM was based on published data regarding accumulation measurements on the gills of fish exposed to copper under various physico-chemical conditions (Playle et al., 1992; 1993) and an established relationship between the gill concentration and the observed effect (MacRae et al., 1999). Based on toxicity data from the literature, BLMs for crustaceans (*Daphnia magna, Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*) were calibrated to predict acute copper and silver toxicity (Di Toro et al., 2001; Santore et al., 2001). By means of the same methodology, Santore et al. (2002) also calibrated an acute Zn-BLM for rainbow trout, fathead minnow and *Daphnia magna*, using a number of relevant toxicity data from literature. The fraction of binding sites that needed to be occupied by the trace metal for causing an effect was calculated by fitting the BLM to existing data derived from metal binding experiments with fish gills. It appears that a certain disadvantage with this method is the assumption that binding constants of competing cations are the same for all species.

De Schamphelaere and Janssen (2002) recently proposed an experimental alternative for developing BLMs, based on the use of experimental effects data from univariate toxicity assays in which one parameter was varied and all other parameters were kept constant. Thereby they could establish a relationship between the observed effect concentration and the presence of a competing cation. Using this methodology, they developed, first, an acute Cu-BLM for *D. magna* (De Schamphelaere et al., 2002) and, secondly, an acute Zn-BLM for the same species (Heijerick et al., 2002a)

Taking into account the much greater complexity of the mechanism of chronic toxicity, the researchers at the University of Gent developed BLMs predicting chronic toxicity to *D. magna* of copper (De Schamphelaere and Janssen, 2003) and zinc (Heijerick et al., 2003), see further details below.

In addition, the same group of scientists set out to investigate the possibilities and limitations of using the BLM concept for modelling copper (De Schamphelaere et al., 2003b) and zinc toxicity to a green alga (*Pseudokirchneriella subcapitata*) (Heijerick et al., 2002b). This species was selected because it was found to be the most sensitive green alga among those tested. The study demonstrated that, with some adaptations, the BLM approach may also be applicable to the assessment of ecotoxicity to algae.

6.3 Application of BLMs

In the first few years of the development of the BLM concept, a series of predictions of the toxicity of copper and silver to freshwater fish were made and compared with results from bioassays conducted in a great variety of test water compositions. It was shown that a wide range of 96h LC_{50} values were obtained simply from adjustments of pH, DOC, alkalinity and the hardness of the test water. The BLM could be used to predict these effects. Input data to the BLM included measured water chemistry (pH, DOC, Ca, Mg, Na, K, Cl, SO_4, CO_3). In ‘expressis verbis’, BLM predicted LC_{50} values representing the amount of trace metal (e.g. copper) necessary to accumulate to lethal BL concentrations. Comparison of BLM results with measured values usually showed an excellent agreement, within a factor of two, of the measured values.

Using the BLM concept, De Schamphelaere and Janssen (2002) developed a method to estimate stability constants (values are given) for the binding of Cu^{2+}, $CuOH^+$, Ca^{2+}, Mg^{2+}, Na^+, and H^+ to the BL, solely based on toxicity data. By using these constants, the authors were able to predict acute Cu toxicity for *D. magna* as a funtion of just these water characteristics. Equilibrium equations for the binding of cations to the BL-sites could be formulated according to:

$$k_{CuBL} = [CuBL^+] / (Cu^{2+}) \cdot (BL^-) \tag{6.1}$$

where k_{CuBL} = stability constant for Cu^{2+} binding to BL sites

By combining mass balances and equilibrium equations for all cations that compete with Cu for binding sites, an equation was obtained for the fraction of the total number of Cu binding sites (ligands) occupied by Cu, which finally determines the magnitude of toxic effects independent of water quality characteristics. From this, 48h-EC_{50} values can be predicted, when Ca^{2+}, Mg^{2+}, Na^+ and H^+ activities are known, but without knowing measured Cu concentrations on the BL. Increases in Ca, Mg, Na (not K) resulted in an increasing 48h-EC_{50} for Cu. In contrast, bioassays showed limitations when the FIAM was used to predict Cu toxicity, since corresponding 48h-EC_{50}s (here as Cu^{2+}) differed by more than a factor of 10. Part of these differences may be explained by the linear relationship observed between EC_{50}s (Cu^{2+}) and Ca^{2+}, Mg^{2+}, Na^+ and H^+ activities and competition between metal ions and cations and hydrogen for binding sites on the biological surface. The fact that also Mg, beside Ca, reduced Cu toxicity suggests species-specific differences. In turn, similar effects of $MgSO_4$ and $MgCl_2$ indicate that inorganic ligands had no effect. The authors also observed differences in EC_{50} when pH was varied and explain this by proton competition at the BL site (linear relation), or by different pH values in the microenvironment of the BL compared to the ambient bulk solution leading to misinterpretation of the Cu-H interaction (De Schamphelaere and Janssen, 2002).

Also $CuOH^+$ toxicity has to be considered, resulting in less Cu^{2+} needed to provoke toxicity at higher pH. A linear regression analysis suggested that indeed $CuOH^+$ co-toxicity rather than proton competition may be the cause for the observed toxicity changes. The good agreement reported by others between the BLM constants of *D. magna* and *P. promelas* (Santore et al., 2001) suggests that daphnid BLs have the same affinity for metals and cations as BLs of fish, which may be incorrect, however, at pH values where $CuOH^+$ co-toxicity becomes relevant. De Schamphelaere and Janssen (2002) found that the BL binding constant, log K_{CuBL} for *D. magna* was 8.02 per M, while the constant for $CuOH^+$, log K_{CuOHBL}, was 7.45 per M.

When the stability constants were included into the BLM computer program designed to predict 48h-EC_{50}s (as dissolved Cu) for the water characteristics measured in the study, the predicted values differed from the observed EC_{50}s only by a factor < 1.5. However, this excellent agreement between predicted and observed toxicity values was found only when tests were performed at pH <8, but not at all for tests performed at pH >8. Additional experiments (De Schamphelaere et al., 2002) demonstrated that this was due to toxicity of the $CuCO_3$ complex, which is the most abundant inorganic copper species at pH >8. The log BL binding constant for this complex was determined to be 7.01 per M.

Later work on chronic copper toxicity to *D. magna* by De Schamphelaere (2003) showed that the log BL binding constants for the hydroxyde and carbonate complexes to gills were higher in chronic tests than in acute tests, 7.75 and 7.38 per M, respectively, indicating that other copper species, in addition to free cupric ions play a substantial role in the manifestation of chronic toxicity to daphnids. Also the effect of pH was more accentuated in the chronic experiments with daphnids compared with the acute tests (cf. review by Van Sprang, 2003).

In an elegant series of experiments Meyer et al. (1999) demonstrated that in laboratory tests, measured concentrations of nickel in gills [Ni $_{gill}$] and calculated concentrations of copper in gills [Cu $_{gill}$] were constant predictors of acute toxicity of Ni and Cu to fathead minnows (*Pimephales promelas*) when water hardness varied up to 10-fold, whereas total aqueous concentrations and free-ion activities of Ni and Cu were not. Thus, the BLM, which simultaneously accounts for (a) metal speciation in exposure water and (b) competitive binding of transition-metal ions and other cations to biotic ligands predicts acute toxicity better than does free –ion activity of Ni and Cu. The LA_{50} value or the "critical BL concentration", i.e. the concentration of metal bound to the fish gill at 2-3 h corresponding to 50% mortality at 96 h, was found to be: for nickel about 250 nmol Ni per g wet weight (ww) and for copper about 12 nmol/g (ww).

Later work, summarized by Di Toro et al. (2001), provided slightly modified values of the "critical BL concentration" in gills of fathead minnows: for Cu – 10 nmol/g (ww), for Ag – 17 nmol/g (ww), and for Ni – 239 nmol/g (ww). In the same paper, critical BL concentrations were given for *Ceriodaphnia dubia* (Cu – 0.19 nmol/g) and for *Daphnia magna* (Ag – 2.26 nmol/g). The fraction of sites at the BL occupied by the trace metal to produce a 50% effect in fish, i.e. the $f_{CuBL}{}^{50\%}$ in the case of copper, was 33% for fish (49% in the case of silver). However, in the crustaceans, the critical fraction of sites occupied by toxicants was only 0.6% in the case of *C. dubia* and Cu, and 6.5% in the case of *D. magna* and Ag. On the other hand, the BL binding constants for both Cu and Ag were almost identical (log values varied only in the range 7.3-7.4 per M) over the three investigated species and so were the site densities (30-35 nmol/g ww) (Di Toro et al., 2001). De Schamphelaere's and Janssen's (2002) determination of BL constants resulted in a value of 39% for the critical fraction of sites at the BL($f_{CuBL}{}^{50\%}$) for *D. magna*, that was occupied by Cu to produce 50% effect.

In the extensive work on zinc (Heijerick et al., 2002a; Janssen, 2003) the BL binding constant, log K_{ZnBL}, was determined at 5.31 for both rainbow trout gills and for *D. magna*. In the case of acute toxicity of Zn, the critical fraction of BL sites occupied by Zn was 14% in the case of rainbow trout, but as much as 42% in the case of *D. magna*. Critical fractions to produce chronic toxicity of Zn (expressed as NOEC values) were 7.4% for rainbow trout and 7.7% for *D. magna*.

Heijerick et al. (2002b) established stability constants (K_{BL}) for the binding of cations to algal cells by using a mathematical approach, which was recently developed by De Schamphelaere and Janssen (2002). By means of adsorption experiments they could show that K^+ is the only cation not significantly changing Zn toxicity. Toxicity tests performed at various pH indicated a pH-dependent change of stability constants, due to competition between H^+ and Zn^{2+}, which was reducing Zn toxicity in a non-linear way. In addition, pH affected also the physiology of the BL (algae). According to the BLM, Zn toxicity to algae can be modelled as a function of key water characteristics. The results of Heijerick et al. (2002b) confirm that the statement that "the binding characteristics of BLs are independent of the test medium charcteristics" is at least not valid for algae. The critical fraction to give chronic effects to algae (EC_{10}) was determined to be 14.3% for the green algal species used.

As a part of the research conducted to produce baseline information for the EU Risk Assessment for Zinc, BLMs were developed to predict chronic zinc toxicity to three standard test organisms, i.e. rainbow trout, *Daphnia magna* and the green alga, *Pseudokirchneriella subspicata*. The work consistently illustrated the importance of bioavailability parameters for chronic toxicity of zinc to rainbow trout, daphnids and algae and demonstrated that changes in zinc bioavailability to aquatic organisms can be quantified and predicted. The differences in toxicity, caused by bioavailability parameters were highest for the alga (a factor of 100), somewhat lower for fish (a factor of 20) and lowest for daphnids (a factor of 4). For the green alga, the pH effect was the most important, while the effects of Ca, Mg and Na were negligible. For the daphnid, hardness and pH appeared to be equally important, whereas for rainbow trout, the effect of Ca was more important than that of pH. The effect of DOC seemed to be most pronounced for the alga, and of similar magnitude for the daphnid and the fish. In spite of these differences, the BLMs for all three organisms were able to significantly reduce the variation in effect concentrations, i.e. chronic effect concentrations were generally predicted within a factor of two from the observed values for all organisms, both when using laboratory waters and waters collected in the field (Janssen, 2003).

It certainly would be wise to keep in mind that, although BLMs seem to function well for organisms such as phytoplankton, zooplankton and fish, which normally take up substances from the surrounding water via well defined biological surfaces, e.g. the cell membrane (in the case of unicellular algae) or the gill membrane (in the case of multi-cellular organisms), thus fitting the GSIM model, it cannot be taken for granted that organisms taking up metals from the surrounding media via other routes will fit any standard BLM. An example clearly demonstrating the existence of such exceptions from the “BLM-rule” was given by Guo et al. (2001), who worked with American oysters (*Crassostrea virginica*). These, and most probably other marine bivalves, which are filter-feeding animals, are capable of concentrating metals from the large volumes of seawater they filter. Since trace metals are often associated with potentially nutritious DOC in the water, it was hypothesized that the uptake of DOC by bivalves may also enhance the uptake of trace metals. It was found that exactly this was the case, especially in the presence of high-molecular-weight DOC at high concentrations, the uptake of metals in the oysters was generally greatly enhanced, possibly due to direct ingestion and digestion of colloidally complexed metals (Guo et al., 2001).

It must be understood, however, that uptake of trace metals into the body tissues of filter-feeding animals does not necessarily result in toxic effects, since metals may be sequestered in not readily available forms in the tissues. In fact, the issue of dietary metal toxicity to filter-feeding organisms such as cladocerans is extremely important. It is obvious that, for example, the BLM developed by De Schamphelaere and Janssen (2003) to predict chronic waterborne copper toxicity to *Daphnia magna* is based on the assumption that there is no contribution of dietary copper to toxicity in the tests used for model development. In order to investigate the validity of this assumption, De Schamphelaere (2003) carried out a great number of chronic tests with *D. magna*, where toxicity was measured at three regimes of exposure to copper : (1) waterborne only, (2) dietary only, and (3) waterborne + dietary. It was found that the 21-d NOEC and EC_{50} for reproduction were nearly identical in the waterborne and in the waterborne + dietary exposure, being 95 and 110 µg Cu/l (as dissolved copper), respectively. From this result it was concluded that the contribution of dietary copper to toxicity was unimportnat and did not interfere with BLM-predicted chronic copper toxicity (De Schamphelaere, 2003). Exposure to dietary copper resulted in an increased copper body burden in the adult daphnids, but no additional toxicity was observed. On the contrary, the dietary exposure route resulted in a 75% increase in growth and reproduction in the highest exposure level. Thus, when cells of green algae, used to feed the daphnids during the tests, were enriched with copper, a significant stimulatory effect was observed in the daphnids.

7 TOXIC AND OTHER ADVERSE BIOLOGICAL EFFECTS OF TRACE METALS

It may be argued that the most important break-through that has occurred in the past decade in the general understanding of toxic and other adverse effects of trace metals on biota in the aquatic and soil ecosystems is the relatively recent acceptance of the bioavailability concept for metals in the environment. Only by accepting the fact that trace metals are not always – or not under all environmental conditions – readily available for uptake into living organisms, is it possible to fully understand and make estimates of the potential risk that metals may pose for man and the environment. Of course, many (but not all) scientists have been aware – for a long time – of the importance of assessing the actual bioavailability of a metal when determining the toxic potential of that metal to any individual species or to a complex community of organisms. However, enlightened scientists have, for many years, not been very successful in making public decision-makers or environmental regulators accept the bioavailability concept when dealing with trace metals in the environment.

One plausible reason for the scientists' lack of success to convince authorities and regulators about the (somehow self-evident) need to consider the fraction of a trace metal that is really mobile, available and reactive, to be able to establish relationships between the harmful concentration and adverse impact, is that scientists have been slow to develop good conceptual models that are both practical and applicable to every-day pollution problems. When environmental chemists, working together with ecotoxicologists, now finally have come up with such readily understandable concepts, such as the Biotic Ligand Models (BLMs) – cf. chapter 6 – and demonstrated how variations in bioavailability of metals in water can be closely related to a variety of water qualities, it has been a relatively rapid process to get acceptance also by regulatory bodies. Such acceptance was achieved at a technical-scientific meeting convened by a EU Committee responsible for the environmental and health effect evaluation of Existing Chemical Substances within the European Union (Eurometaux, 05/2003). At the same meeting, major progress was also made with regard to the bioavailability of trace metals in another environmental compartment, i.e. in aquatic sediments. It was approved that bioavailability and toxicity of metals in sediments could be assessed by using the SEM/AVS concept (cf. section 5.4.3).

Just by introducing these two new tools, it is now suddenly possible to talk about toxic effects on biota in aquatic and soil ecosystems by means of a much clearer language, facilitating the understanding of some rather complex phenomena in nature. Before, it was not possible to describe the chronic toxic concentrations of, for example, zinc to freshwater fish without giving long lists of results from hundreds of bioassays carried out in an array of different water types, from relatively uniform laboratory waters to surface waters originating in different geological regions. It was not an easy task, for example, to grasp the rather confusing data base, showing that zinc could exert chronic toxicity (30-d EC_{10} values) to one single life stage in one specified fish species, i.e. juvenile rainbow trout, at concentrations ranging from 38 to 900 µg Zn/l.

7.1 Toxicity to aquatic organisms in the water column

In recent years, it has – again and again – been recognized that the toxicity, e.g. the acute median lethality, LC_{50}, of a trace metal to a single aquatic species can vary widely between different bioassays. This has become a problem, because results of toxicity tests are often used to establish Water Quality Criteria or as inputs into environmental risk assessments, and with variable toxicity data it is difficult to apply criteria based thereon with any certainty.

Therefore, environmental chemists and ecotoxicologists have concentrated, over the last few years, on careful examinations of the causes for the variation in toxicity and then, the relative importance of the various toxicity-modifying factors has been quantified. The variation in trace metal toxicity is directly related to variations in their bioavailability and speciation which, in turn, are largely governed by water hardness, pH and the concentration of organic ligands.

In this sub-chapter examples are given of both the extremely large biological species-related variation in acute and chronic toxicity data for copper in freshwater (LC_{50}-values ranging from 5 to 20,000 µg/l, and chronic thresholds, NOEC values, from 2.0 to 338 µg/l, as dissolved Cu) and the water hardness-related wide variation in LC_{50}-values within one single species (of fish). Recently reported data indicate that in tests with invertebrates and microalgae, dissolved organic carbon (DOC), followed by pH, is affecting chronic copper toxicity to a much greater extent than water hardness. The variability in aquatic copper toxicity can be reduced to about a factor of 2 when the organisms' toxic response to copper in the water is *estimated by means of the Biotic Ligand Model (BLM). By this method it was demonstrated that the conditional binding constants of cupric ions to the biotic ligands (BL) in gill tissue of both fish and daphnids were fairly constant (log $K_{CuBL} = 7.4 - 8.0$), and that difference in sensitivity between species was reflected by the fraction of binding sites in gills that must be occupied by metal to produce 50% mortality (varying from 0.6 to 40% in the case of Cu). Other copper species, such as $CuOH^+$ and $CuCO_3$, do contribute to copper toxicity, since they exhibit fairly strong binding to gill BLs.*

Compilation of aquatic toxicity data on nickel has provided evidence that algae and, especially, invertebrates are much more sensitive to nickel exposure than fish, among which rainbow trout larvae appear to be the most sensitive with a 96-h LC_{50}-value of 50 µg/l in soft freshwater. Nickel toxicity to fish is greatly affected by the age of the fish followed by the DOC content and hardness of the water.The lowest chronic effect value recorded for nickel was found in a test with a marine crustacean, 22 µg/l. However, based on BLM predictions of acute toxicity, the sensitivity of freshwater fish to nickel was about 20-25 times lower than that to copper.

The lowest recently reported value for chronic zinc toxicity to fish in very soft freshwater is in the range 16–27 µg/l, a level corresponding to the range given in the previous zinc monograph (Landner and Lindeström, 1998) for "maximum tolerable concentrations" of zinc in soft inland waters, 15–25 µg Zn/l. The conditional stability constant for binding of zinc to the BL (log K_{ZnBL}) was estimated at 5.31 and the fraction of binding sites occupied by zinc to produce 50% mortality in acute exposure of rainbow trout was found to be 14%. The fraction of binding sites corresponding to a "no-effect-level" on chronic exposure was determined to be in the range 5.1–7.7% in rainbow trout and Daphnia magna*. In a series of predictions of safe zinc concentrations (or no-effect-concentrations) on chronic exposure in European inland waters, based on the authentic, prevailing water qualities, the results were: 120–1,080 µg/l for rainbow trout, 110–760 µg/l for daphnids and 23–100 µg/l for green algae. Variations are due to varying concentrations of organic matter, prone to form non-bioavailable complexes with zinc.*

7.1.1 Copper

7.1.1.1 Sensitivity to copper of different aquatic organisms

Because copper is an essential trace metal, several groups of aquatic organisms have developed strategies for regulating internal copper concentrations (cf. e.g., Brix et al., 2001). These physiological strategies range across a continuum from active regulation (e.g., excretion of excess copper or limitation of net uptake) to storage, i.e. sequestering excess copper in forms that are either metabolically available (e.g., metallothioneins) or unavailable (e.g., calcium phosphate granules) (Philips and Rainbow, 1989; Rainbow and White, 1989; Depledge and Rainbow, 1990). It is also necessary to consider the permeability of membranes, that can influence the sensitivity to metals. This is demonstrated by the fact that some organisms can adapt to elevated metal levels in the surroundings by reducing their membrane permeability. Finally, allometric considerations may be important, such as the amount of permeable membrane relative to absolute body size.

The capacity of organisms to regulate internal levels of copper – and other trace metals – and thereby respond to toxic effects of the metals, may be jeopardized if the organism is exposed to simultaneous additional environmental stress. For example, when the gammarid amphipod *Paramoera walkeri* (living in coastal Antarctic waters) was simultaneously exposed to UV-B radiation and food shortage, its sensitivity to copper increased by more than 30-fold. The added environmental stress caused by UV-B radiation obviously reduced the general fitness of the amphipods, and food shortage further increased their sensitivity to copper. The finding indicates that energy is required by the amphipods to cope with exposure to copper (Liess et al., 2001). These findings are also in agreement with the fact that starving fish get a reduced capability of excreting copper via the bile. For example, starved yearling roach showed much higher accumulation of copper in the liver than fed fish, suggesting that food-deprived fish lack the ability to regulate transfer of copper within the body (Segner, 1987).

Another important biological phenomenon that can influence the general level of copper sensitivity in aquatic organisms is the social interactions in a population. In a study of the effects of copper on the behaviour of stream-dwelling salmonid fish, as well as the effects of social behaviour on copper uptake, it was found that the salmonids' characteristic social behaviour was not influenced by sub-lethal waterborne exposure up to 30 µg Cu/l. However, the social status of a fish determined its copper uptake, so that subordinate fish accumulated more copper (and sodium) from the water than dominant fish (Sloman et al., 2002).

It has been repeatedly mentioned in this report, e.g. in chapter 6, that water hardness is a major factor determining the speciation and, thus, the bioavailability and toxicity to fish of waterborne copper. In order to give a direct impression of to what extent water hardness will cause variation in acute toxicity to fish, it may be pertinent to cite some data compiled by Hansen et al. (2002), showing rainbow trout LC_{50} values (96 h) measured at different water hardness levels on juvenile fish with body weights of <5 g. Some data in very condensed form are shown in Table 7.1.

Table 7.1. LC_{50} values (96 h) for dissolved copper (µg Cu/l) measured with larval rainbow trout (body weight <5 g) at different levels of water hardness. Compiled from older studies by Hansen et al., 2002.

Hardness, mg/l as $CaCO_3$	LC_{50} values (96 h), µg Cu/l
9	2.8
25	17 – 28
31 – 41	3.4 – 36
90 – 102	17 – 48
100	36 – 93 *
83 – 274	
220	100 – 310 *

* results from experimental work by Hansen et al., 2002

It is clear from the above table that acute toxicity of copper to fish varies with water hardness. Therefore, it is important to keep in mind that when copper toxicity data is expressed in this classical way, as concentrations of dissolved copper in water, the water quality (e.g. the hardness) of the water resources to be assessed must be considered. In the Scandinavian countries, lakes are quite often characterized by medium, low, or even very low water hardness, meaning that numerical values in the range 10-50 mg/l as $CaCO_3$ are quite common (Regoli, 2003). Only in the south-eastern part of Sweden (except the southernmost province of Scania), hardness levels of 50 mg/l and higher are usually found. This means that the acute toxicity data for rainbow trout that was normalised to 50 mg/l hardness (39 µg Cu/l, Table 7.2) would in stead fall in the range 3.4 – 36 µg Cu/l in large parts of the Swedish inland waters.

The data summarized in Table 7.1 also indicates that there are other causes of toxicity data variation, in addition to water hardness. For example, the hardness interval 31 – 41 mg/l corresponded to LC_{50} values (96 h) as low as 3.4, 8.1 and 14 μg Cu/l, although the lowest LC_{50} value at the hardness levels 25 and 90 – 102 was 17 μg Cu/l. No good explanation of these variations in acute toxic values was given in the paper by Hansen et al. (2002). However, it is also well-known that organic copper complexes are readily formed in freshwater and that these are generally not bioavailable nor toxic. Thus, the explanation of the variations shown in Table 7.1 may well be that concentrations of organic ligands in the different tests show a wide, but possibly undetected, variation.

De Schamphelaere (2003) has shown that aquatic organisms other than fish have a somewhat different pattern of reaction to water hardness as a modifyer of copper toxicity. In, for example, *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata* water hardness did not significantly affect chronic copper toxicity. On the other hand, it was demonstrated that DOC explained about 60% of the variability and that pH explained about 15% of the variability in the chronic toxicity values in these test organisms.

In another paper, the copper complexation capacity was determined in a range of final effluents from sewage treatment plants and in the receiving waters upstream and downstream of the discharge point and the variation in copper toxicity to *Daphnia magna* was recorded (van Veen et al., 2002). The studied sewage effluents contained copper-complexing ligands in a three- to eight-fold excess of total dissolved copper, and the ligand concentrations were found to correlate strongly with DOC concentrations in the effluents. The toxicity of copper to the daphnids was greatly modified by the sewage-derived organic ligands. In the most extreme case, the tolerance of *Daphnia* to dissolved copper was quadrupled, in close relationship with the complexing capacity and the DOC concentration of the test solution.

Davies et al. (1998) developed a bioassay with Cu-sensitive bacteria isolated from tropical river water to determine Cu bioavailability. The strong linear correlation ($r=0.93$, $p<0.005$) observed between measured EC15 values and the Cu complexation capacity of the river water as determined by anodic stripping voltammetry (ASV) confirmed again the role of natural organic matter to mitigate Cu toxicity and that Cu bioavailability in freshwaters is reduced by natural organic compounds.

In their comprehensive work to develop species sensitivity distributions for different taxonomic groups of freshwater aquatic animals, Brix et al. (2001) used copper as an example. They have compiled a great number of data on acute copper toxicity from the U.S. EPA copper toxicity database and from other sources. Acute data for 86 different freshwater animal species were listed, where LC_{50} values were normalised to a hardness of 50 mg/l (as $CaCO_3$) using the U.S. EPA's equation for hardness normalisation (U.S. EPA, 1985). Moreover, chronic data (also normalised to a hardness of 50 mg/l) were compiled for 17 species. All toxicity data are from laboratory experiments and are apparently expressed in terms of total dissolved copper. Just to give an impression of the distribution in sensitivity, acute and chronic data are presented for those species on which measured chronic data is available (Table 7.2).

Table 7.2. Examples of acute and chronic toxicity data regarding dissolved copper (µg/l), normalised to a water hardness of 50 mg/l, for freshwater animals belonging to different taxonomic groups. After Brix et al., 2001.

Main group	Species	SMAV*	SMCV**	ACR***
Cladoceran	*Daphnia magna*	18.1	7.7	2.4
d:o	Other daphnids	5.2 – 69		
Amphipod	*Gammarus pseudolimnaeus*	22.1	6.7	3.3
Rainbow trout	*Oncorhynchus mykiss*	39	20.6	1.9
Brook trout	*Salvelinus fontinalis*	110	8.3	13.3
Bluntnose minnow	*Pimephales notatus*	72.2	(2.8)	25.8
Fathead minnow	*P. promelas*	133	11.2	11.9
Bluegill sunfish	*Lepomis macrochirus*	1130	32	35.3
Snail	*Physa integra*	43.1	11.9	3.6
d:o	*Campeloma decisum*	1880	11.9	158
Caddisfly	*Clinostornia sp.*	6200	18.2	340

* Species Mean Acute Value – geometric mean of LC_{50} values for a given species

** Species Mean Chronic Value – geometric mean of the mean of LOEC and NOEC for a given species

*** Acute-Chronic Ratio

The 86 freshwater species for which (quality controlled) acute test data was available showed a range in normalised LC_{50} values for dissolved copper from 5.2 to 20,000 µg/l. The 17 species that exhibited high quality data on chronic toxicity to copper showed a much smaller variation, 2.8 – 65.6 µg/l (Brix et al., 2001). If one "outlier", the northern pike, *Esox lucius*, is excluded, the range is reduced to 2.8 – 33.4 µg/l. The very low estimate of chronic toxicity to bluntnose minnow, 2.8 µg Cu/l, may furthermore merit a special comment. On closer examination of the original paper (Horning and Neiheisel, 1979), it turns out that the lowest tested concentration was 18 µg/l, which makes the estimate of the SMCV at 2.8 µg/l very unreliable. Moreover, the estimate of chronic toxicity to the fathead minnow (same genus as bluntnose minnow), was based on 5 tests and gave a geometric mean of 11.2 µg/l. The acute values for the two species were not as far apart (72 µg/l and 133 µg/l) as the chronic values. It is known that differences in the susceptibility of individual fathead minnows – and perhaps bluntnose minnows – to Cu are genetically determined to a large extent. Genetic differences in minnow susceptibility to Cu are possibly coded by genes producing proteins involved in ionoregulation and/or accumulation and sequestering of Cu (cf. Kolok et al., 2002).

Among invertebrates, the cladoceran taxonomic group clearly is the one most sensitive to Cu of those investigated, while insects are, on average, the least sensitive one. For fish, on the other hand, it was not possible to identify any trend in relative sensitivity with respect to feeding guild or phylogenetic relationship. There was, however, a general trend towards higher sensitivity of temperate cold-water species, followed by temperate warm-water species, which in turn appeared to be more sensitive than tropical species (Brix et al., 2001).

In order to complement the database on chronic toxicity of Cu to freshwater animals, such data was estimated from the SMAVs, using a variable acute-chronic ratio (ACR), according to the following equation:

$$\mathrm{ACR} = 10^{0.786 \times \log \text{Acute Value} - 0.582} \qquad (7.1)$$

This approach was chosen because it was found that when chronic data was estimated with a variable ACR, the correspondence with measured data was much better for a variety of species, than when using a constant ACR (Brix et al., 2001). It was also demonstrated that the distribution of measured chronic data was much steeper than the acute effects distribution, which is reflected in Table 7.2. These results suggest that there may be a chronic threshold for Cu related to the ability of organisms to regulate this essential element.

Later literature surveys made it possible to increase the database of observed high-quality chronic NOECs for Cu in the aquatic environment : Delbeke et al. (2003) compiled 126 NOECs from 21 species and found them to range between 2 and 338 µg Cu/l as dissolved Cu. In this context, it may be pertient to mention that in a recent paper (Baldwin et al., 2003), it was shown that when concentrations of total dissolved Cu were increased by 2.3-3.0 µg/l over background concentration, Cu was clearly toxic to the olfactory nervous system in coho salmon (*Oncorhynchus kisutch*). The inhibitory effects of copper were dose-dependent and they were not influenced by water hardness.

In their recent paper, Delbeke et al. (2003) also presents an exercise of estimating a provisional PNEC value for Cu in freshwater, based on the species sensitivity distribution, the chronic NOECs, of fish and invertebrates, compiled from the literature. When the NOECs were normalised to the 5th percentile of pH, water hardness and DOC (taken from the comprehensive physico-chemical water quality database for European inland waters) by means of the chronic Cu BLM, developed for gill-breathing organisms, the combined outcome in terms of a normalised PNEC was found to be 8 µg Cu/l as dissolved Cu.

7.1.1.2 Toxicity of copper estimated by means of BLMs

In the previous section, a certain number of examples were given, all supporting the view that the toxicity of copper to freshwater aquatic organisms is dependent on a variety of ambient water chemistry parameters. This view is further strengthened by data compiled by Van Sprang (2002) showing a variation in LC_{50} values for one single fish species (*Pimephales promelas*) from 10 to 1000 µg Cu/l when the acute toxic concentration is expressed as dissolved aqueous copper. In a similar survey presented by Di Toro et al. (2001), the LC_{50} values for larval stages of the same fish species ranged between 6.0 and 1560 µg Cu/l.

This variability in toxicity due to variation in water chemistry was further confirmed and demonstrated in the work by de Schamphelaere and Jansen (2002), where they developed a BLM predicting copper toxicity for *Daphnia magna.* As a part of their efforts to estimate biotic ligand constants, these authors conducted a series of acute toxicity tests with *D. magna*, in order to assess the variation in 48-h $EC50_{Cu2+}$ as a function of the chemical activity of calcium, magnesium, sodium, potassium, hydrogen and hydroxide ions. In each group of tests, one among six different water quality parameters was systematically varied while all the others were kept constant and the toxic response (EC50) was measured. In Table 7.3, the variation in EC50 values, expressed as total dissolved copper, is shown as a function of the variation in the different chemical activities.

Table 7.3. Variation in toxic response (EC50) in Daphnia magna, expressed as µg/l of total dissolved copper, when each of six chemical activities (in mM) in the test solution was changed. After De Schamphelaere and Janssen, 2002.

Chem. activity modified	Range 1, mM	Range 2, mM	Variation in EC50, µg Cu/l
Ca	0.25 – 4.0		5.2 – 12.1
Mg (1) and SO_4 (2)	0.25 – 5.0	0.25 – 5.0	3.7 – 17.5
Mg (1) and Cl_2 (2)	0.25 – 4.0	1.08 – 8.6	6.8 – 14.6
Na	1.08 – 15.1		5.8 – 21.2
K	0.08 – 2.0		6.2 – 6.9
pH	5.98 – 7.92		5.6 – 52.4

Modification of each of the parameters, except potassium, produced quite strong variation in toxic response of the test animals. In relative terms, manipulation of the pH caused the strongest variation in EC50 values for copper. It should be noted, however, that the DOC concentrations in these tests were assumed to be constant (0.14 mg DOC/l) and to have the same metal complexation properties as natural organic matter. Thus, in an authentic, natural surface water, the DOC – as a cause of variation in EC50 values for copper – should be added to the causes of variation related to the above investigated inorganic parameters. As a matter of fact, it was later clearly demonstrated that DOC is the most important water quality factor among all those affecting the chronic copper toxicity to *Daphnia magna* (De Schamphelaere, 2003).

As it has been repeatedly mentioned before, the great variability in toxic response to copper by aquatic organisms, depending on the prevailing water quality, can be almost eliminated by deriving the toxic response by means of a BLM. Van Sprang (2002) showed that the BLM-predicted EC50s differed from the observed EC50s by a factor of less than 2, while De Scahmphelaere and Janssen (2002) found this difference to be a factor of less than 1.5.

In the assessment of acute toxicity by means of BLM, the biotic ligand for fish is the gill. What is needed, thus, is to predict the amount of metal accumulated at the surface of the fish gill in order to predict metal toxicity to the fish (Di Toro et al., 2001). When juvenile rainbow trout or fathead minnows are exposed to dissolved copper, a relatively rapid increase occurs over background levels of copper bound to the gills. This rapid initial increase takes place in a few hours to a day (Playle et al., 1992). It is believed that the rapid initial increase in gill copper reflects binding to receptor sites at the gill surface that control the ionoregulatory processes of the fish. The next step in predicting acute toxicity is to quantify the relationship between mortality of the fish after 120 h of exposure and copper concentration on the gill after 24 h of exposure.

MacRae et al. (1999) performed a series of experiments with juvenile rainbow trout, using a constant total dissolved copper concentration (10 µg/l), and the gill copper concentration was regulated by adding different organic ligands with varying affinities for copper to the test water. The gill copper concentration that caused 50% mortality was estimated to be 22 nmol/g wet weight (ww). The background gill copper concentration (at no mortality) is approximately 12 nmol/g ww, a value that corresponds well with the gill background in fathead minnow, measured by Playle et al. (1992) in the absence of added copper. It was provisionally concluded that the gill copper LC_{50} (defined as the LA_{50}, median lethal activity, value) should be approximately 10 nmol/g ww (the difference between the measured value and the background) for both fish species.

Further work by Di Toro et al. (2001), using a range of DOC, hardness and pH conditions in the tests, confirmed that the LA_{50} value for fathead minnow averaged 5 to 12 nmol/g ww. This finding is consistent with the previously demonstrated similar sensitivity of fathead minnow and rainbow trout to copper. It is also consistent with the finding that the two fish species accumulate copper on their gills in a similar manner and suggests that binding constants for metal-gill interactions for one species can be generalised to other fish species. The copper-gill BL binding constants for rainbow trout (7.5) and brook trout (7.2), found by MacRae et al. (1999) are quite close to the value for fathead minnow (7.4) found by Playle et al. (1993). Thus, the BLM can explicitly account for variation in toxicity resulting not only from changes in hardness, but also from site-specific variations in DOC, pH and alkalinity.

Although the BLM has turned out to be an excellent tool to handle the great variation in acute toxicity values caused by the variable conditions for trace metal speciation and complexation in the water, and also to consider the competition between different ions for binding sites at the BL (cf. chapter 6), there is – of course – still a variation in toxicity due to differences in sensitivity between the biological species. This may be illustrated by the compilation of “critical BL concentrations” or LA_{50} values for different organisms shown in Table 7.4.

Table 7.4. BL binding constants, log K_{MeBL} (M^{-1}), site densities in gills (nmol/g ww) and critical BL concentrations or LA_{50} values (nmol/g ww), and fraction of binding sites occupied by metal to produce 50% mortality for copper, silver and nickel in various organisms. Sources: Di Toro et al., 2001; De Schamphelaere and Janssen, 2002 ; De Schamphelaere, 2003.

Species	Metal	log K_{MeBL}	Site density	LA_{50}value	$f_{MeBL}^{50\%}$
Rainbow trout	Cu	7.5	(30)*	10	(0.33)*
Fathead minnow	Cu	7.4	30	5 – 12	0.21***
	Ag	7.3	35	17	0.49
	Ni	n.r.**	n.r.**	239	n.r.**
Ceriodaphnia dubia	Cu	7.4	30	0.19	0.006
Daphnia magna	Cu	8.0	(30)*	(12)*	0.39, 0.47
	Ag	7.3	35	2.3	0.065

* figures in brackets are provisional estimates made by authors of the present report

** n.r. = not reported

*** constant from Santore et al. (2002)

It should be noted that the latest constants calculated for acute toxicity of copper to *Daphnia magna* are based on the observation that two copper species in addition to cupric ions are bioavailable and can produce toxic effects in the animals, viz. $CuOH^+$ and $CuCO_3$. The log K_{MeBL} constants for these complexes are estimated at 7.32 and 7.01, respectively, i.e. clearly lower than the constant for cupric ions, 8.02 (De Schamphelaere, 2003).

Table 7.4 indicates that binding constants, log $K_{MeBL,}$ for metal-gill interactions are quite similar between different biological species and for, at least, copper and silver. (It should be noted, however, that the log K_{ZnBL} for acute Zn-BLM to *Daphnia magna* was found to be 5.31 (Heijerick et al., 2002a)). Also the site density, i.e. the total binding site density of the biotic ligand (e.g. nmol of available sites per gram of tissue) appears to be quite similar for different species and metals. The variation in sensitivity between organisms and metals is primarily reflected in the critical concentration producing mortality, i.e. the LA_{50} value. This value shows a certain variation between the fish and the tested crustaceans. The fraction of binding sites needed to be occupied by the actual metal to cause 50% mortality is, consequently, also a variable.

In the development of their predictive copper toxicity model for green microalgae, De Schamphelaere et al. (2003b) demonstrated that DOC concentration and origin as well as pH of the test water had a significant effect on copper toxicity to the algae. The model was also validated by using copper-spiked European surface waters.

However, most of the BLM parameters discussed above have been determined in laboratory tests in waters with very low concentrations of DOC (or NOM, natural organic matter). It is therefore important to consider to what extent predictions of toxicity in natural waters may be biased by this lack of realistic experimental conditions. Just to give a few examples, some results given by Richards et al. (2001) may illustrate the issue: Copper was found to bind to NOM about 50 times better than to rainbow trout gills (conditional binding constants of log $K_{Cu\text{-}NOM} = 9.1$ and log $K_{Cu\text{-}gill} = 7.4$), while cadmium bound about 16 times less well to NOM than to gills (log $K_{Cd\text{-}NOM} = 7.4$; log $K_{Cd\text{-}gill} = 8.6$). Thus, NOM in the experiments conducted by Richards et al. (2001), was able to keep copper, but not cadmium, from binding to the gills. Moreover, it was found that silver binds about 10 times more strongly to the rainbow trout gills than to NOM (log $K_{Ag\text{-}gill} = 10.0$; log $K_{Ag\text{-}NOM} = 9.0$), while cobalt showed the same binding strength to the two kinds of ligands (log $K = 5.1$).

The conditional stability constants for sorption of copper to the marine alga *Emiliania huxleyi* were determined by Teresa et al. (2001) both with regard to extracellular adsorption (K_{CuSe}) and intracellular uptake (K_{CuSi}). The value of log K_{CuSe} was 12.3 and that of log K_{CuSi} was 11.0, and the constant for extracellular adsorption was compared to the corresponding values (from the literature) for two other algae, one marine (*Dunaliella sp.*) – 8.8, and one freshwater species (*Scenedesmus sp.*) – 11.1.

An interesting observation made during the experiments with *E. huxleyi* was that copper dissolved in natural coastal seawater was taken up very rapidly into the algal cells: after 10 min. exposure, some 80% of the equilibrium amount of copper in the cells was already intracellular, while the process of external adsorption was somewhat slower. Moreover, Teresa et al. (2001) also determined the conditional stability constants for copper bound to either the exudates excreted by the algal cells as a result of copper exposure or to the organic ligands occurring in the seawater prior to copper addition. In both cases they found that these constants were practically of the same magnitude (12.2 – 12.3) as the constants for extracellular adsorption of copper to algal cells. The production of exudates from the cells was quite fast and after 10 min., the molar concentration of organic ligands in the water was close to the molar concentration of copper. It was therefore concluded that the ligands present in the seawater, mainly those released by the cells, can compete efficiently for copper with the *E. huxleyi* cell sites.

7.1.2 Nickel

Most aquatic organisms are less sensitive to nickel exposure than to copper exposure, which is reflected by higher numerical values of the LC_{50}s, but also of the measures of chronic toxicity. Toxicity of nickel in natural waters varies widely as a function of pH and hardness and – in particular – of DOC concentration.

Spencer (1980, ref. in IPCS, 1991) has reported that nickel at concentrations of 50 – 100 µg/l (as dissolved Ni) inhibits the growth of algae, though some species may be more tolerant. Especially green algae were indicated to be sensitive to nickel exposure at 100 µg/l, while blue-greens appeared to be less sensitive.

Madoni (2000) measured the acute toxicity of nickel ($NiCl_2·6\ H_2O$) to freshwater ciliates and found toxic concentrations ranging from 0.17 mg/l (*Spirotomum teres*) and 0.36 mg/l *(Paramecium bursaria*) to 7.7 mg/l (*Euplotes patella*).

In tests with *Daphnia magna*, a 64-h LC_{50} value of 320 µg/l was recorded at a water hardness of about 100 mg/l $CaCO_3$, while another species, *D. hyalina*, had a 48-h LC_{50} value of 1.9 mg/l at a lower water hardness, 58 mg/l (IPCS, 1991). Impairment of reproduction in *Daphnia* was recorded at nickel concentrations as low as 30 – 95 µg/l in water with pH 7.74 and a hardness of about 45 mg/l $CaCO_3$ (Biesinger and Christensen, 1972).

The sensitivity of fish to nickel varies considerably among species, but 96-h median lethal concentrations in soft freshwater generally fall within the range of 4 – 20 mg/l, i.e. the sensitivity of fish to acute effects of nickel is much lower than that of invertebrates and algae (IPCS, 1991). However, there are life stages in fish which show considerably higher sensitivity to nickel exposure; e.g. when eggs and larvae of rainbow trout were exposed at a water hardness of 100 mg/l $CaCO_3$ (from fertilization to 4 days after hatching), the LC_{50} value was found to be 50 µg/l (Birge and Black, 1980). In these experiments, it turned out that rainbow trout was significantly more sensitive to nickel exposure than any of the other about 10 different fish species tested (IPCS, 1991).

Recent work by Hoang et al. (2004) with fathead minnows of different age (<1-d old and 28-d old) under varying regimes of water hardness, pH, alkalinity and natural organic matter (NOM) demonstrated the following : The toxicity of Ni was inversly related to water hardness at $CaCO_3$ levels between 20 and 150 mg/l. In the absence of NOM, Ni toxicity increased as pH increased at high hardness and alkalinity. The fry (<1-d old) were more sensitive to Ni than the 28-d old fish : the difference in sensitivity was 12-fold at low hardness and alkalinity, but 5-fold at higher hardness and alkalinity (100 and 400 mg/l, respectively). The presence of NOM (10 mg DOC/l) reduced Ni toxicity by up to 50%, but this effect was saturated above DOC at 5 mg/l. The BLM that was developed gave results suggesting that the model could be improved by considering $NiCO_3$ to be bioavailable.

As shown in Table 7.4 above, the relative sensitivity of fathead minnows to toxic effects of trace metals, expressed as critical concentration of metal bound to the biotic ligand in the gills, is much lower for nickel than for copper or silver. The LA_{50} value for nickel was given as 239 nmol/g ww, and for copper it is about 10 (5 – 12) nmol/g ww. Based on this data, the sensitivity of this fish species to nickel appears to be about 20 – 25 times lower than its sensitivity to copper.

In tests with three marine organisms, where both acute and chronic toxicity data were obtained, it turned out that a fish (*Atherinopsis affinis*) had much lower sensitivity to nickel (acute – 27 mg/l; chronic – 4.3 mg/l) than a mollusk (*Haliotis rufescens* – the red abalone), with acute and chronic values at 150 µg/l and 26 µg/l, and a crustacean (*Mysidopsis intii*), exhibiting an acute toxicity, i.e. 96-h LC_{50}, of 150 µg/l and a chronic toxicity of 22 µg/l (Hunt et al., 2002). These chronic values, recorded in two marine invertebrates, appear to be among the lowest observed effect concentrations so far reported for nickel.

7.1.3 Zinc

7.1.3.1 Sensitivity to zinc of different aquatic organisms

An overwhelmingly great fraction of published data on zinc toxicity to aquatic organisms unfortunately suffers from poor quality. For example, many of the older studies suffer from such great deficiencies in experimental design and data presentation that it is doubtful whether the results can be regarded as scientifically well-founded (cf. L & L – Zn, Appendix 1). In fact, it is only in the relatively recent ecotoxicological literature on the aquatic toxicity of Zn that information necessary for reasonable interpretation of the data is given. Such crucial information would include, in addition to test duration, toxic end-point(s) assessed, mortality in control groups and other biological background data, a minimum number of physical/ chemical data, such as temperature, pH and measured Zn concentration in the test solution, as well as water hardness, DOC and the possible occurrence of synthetic complexing agents.

Within the scope of this update of the previous monographs on metals, it is not our intention to try to make a comprehensive review of the very abundant new literature on Zn toxicity. In stead, we would like to refer the reader to the summaries of aquatic toxicity data in the recent "Environmental Health Criteria" on Zn (IPCS, 2001) and to the comprehensive compilation of aquatic toxicity data on Zn made as a part of the EU Environmental Risk Assessment (RA) on Zinc (RIVM, 1999). Research carried out in conjunction with this RA has indicated that the sensitivity of the toxic response of a test organism to an essential metal like Zn is a function of the concentrations of essential elements in which it was cultivated. For example, test organisms reared under conditions where the dissolved Zn concentration was <2 μg/l exhibited a mean NOEC of 36 μg Zn/l, while test organisms reared at >2-<50 μg Zn/l had a mean NOEC of 100 μg Zn/l as dissolved Zn (Waeterschoot et al., 2003). It was furthermore demonstrated that a dissolved Zn concentration of 17 μg/l would provide protection of 95% of the tested aquatic species in the water qualities typical for lowland river systems in Europe.

In the zinc monograph (L & L – Zn) the maximum tolerable concentration (MTC) of zinc in soft inland waters was set at 25 μg dissolved Zn/l to achieve 95% protection of all endpoints in the aquatic ecosystem, and at 15 μg Zn/l to achieve close to 100% protection.That the regional, continental European PNEC value would be at a level of 15-25 μg Zn/l is further supported by a recent report by Woodling et al. (2002), where the authors have determined the chronic toxicity of zinc to the mottled sculpin (*Cottus bairdi*) in soft water (46.3 mg/l as $CaCO_3$) to 27 μg/l (LOEC) and 16 μg/l (NOEC), a lower toxic zinc concentration than observed for any other fish species tested to date (according to the authors)..

What is really new in the field of research on aquatic toxicity of zinc is the development of BLMs predicting acute and chronic zinc toxicity to standard test organisms, rainbow trout, *Daphnia magna* and a green alga. In this work, the various causes of variation in the toxicity values were identified and quantified so that prediction of chronic effect concentrations could be made within a factor 2 of the observed effect concentrations, not only for laboratory waters but for natural surface waters as well. These new advances in zinc ecotoxicity research will be briefly presented in the following section.

7.1.3.2 Toxicity of zinc estimated by means of BLMs

As mentioned in chapter 6 and in section 7.1.1.2, the BLM concept was originally developed by Di Toro et al. (2001) using the conditional stability constants for competing cations (e.g. Ca^{2+}, H^+) and trace metals for binding to biotic ligands on fish gills, derived from metal binding experiments with fish gills (Playle et al., 1993). The fraction of binding sites that needs to be occupied for causing an effect was calculated in the early work by fitting the BLM to existing literature data (Santore et al., 2002). However, an improved methodology was developed by De Schamphelaere and Janssen (2002), where they used experimental effects data of univariate toxicity assays, in which one parameter was varied and all other parameters were kept constant. This approach was successfully applied to the development of acute BLMs for copper and zinc with *D. magna*, and later on, it was used to develop chronic BLMs for copper and zinc with rainbow trout, *D. magna* and the green alga, *Pseudokirchneriella subcapitata* (De Schamphelaere et al., 2003a ; b ; Heijerick et al., 2003).

The main purpose of this work was to test the potential of the BLMs to predict copper and zinc speciation, complexation, bioavailability and chronic aquatic toxicity over a relevant range of water chemistry conditions as existing in European inland surface waters.

The importance of various bioavailability modifying factors (water quality parameters) for chronic zinc toxicity to the three test species was quantified and the relative importance of each of these is summarized in Table 7.5. The toxic end-point used for rainbow trout was mortality, for daphnids mortality and reproduction impairment, and for the green alga, growth rate deviating from that of the control.

Table 7.5. Importance of zinc bioavailability modifying factors for toxicity variation: number of times the lowest and highest toxicity differed in a series of univariate chronic toxicity tests with zinc for rainbow trout (RT), *D. magna* (DM) and the green alga *P. subcapitata* (PS). After De Schamphelaere et al., 2003a.

Parameter	RT	DM	PS
Calcium	≈ 10	3 – 4	--
Magnesium	3 – 4	2 – 3	2
Sodium	2	1.5	--
pH (H^+)	2 – 3	3 – 4	>20
DOC	≈ 5	1.3-1.5	14
Overall, EC_{50}	18	3.5	63
d:o , NOEC	28	3.5	127

As shown in Table 7.5, the changes in zinc bioavailability to aquatic organisms can be quantified and predicted. The toxicity differences caused by bioavailability parameters were highest for algae (a factor of 130), and lower for fish (factor 30) and daphnids (factor 4). For algae the pH effect was the most important, but for fish, the effect of calcium was greater than that of pH. In spite of these differences, BLMs for the three organisms were able to significantly reduce the variation; i.e. the chronic effect concentrations were generally predicted within a factor of 2 from the observed values for all three organisms. Thus, it was concluded that the BLMs developed in the study conducted by Schamphelaere et al. (2003a) could be used as efficient tools to reduce the toxicity variation, due to differences in zinc bioavailability, from a factor of more than 100 to a factor of 2, in both laboratory and field waters.

The conditional stability constant for binding of zinc to the BL, log K_{ZnBL}, was assumed to be equal to the one reported for the acute Zn-BLM for *Daphnia magna* by Heijerick et al. (2002a), i.e. 5.31, and equal in all three organisms. The fraction of binding sites occupied by zinc to produce 50% mortality in **acute** exposures, the $f_{ZnBL}^{50\%}$, was found to be 14% in the case of rainbow trout and about 42% in the case of *D. magna*. The corresponding *f* values in the **chronic** exposures are shown in Table 7.6, together with the chronic toxicity levels, recalculated as µg dissolved Zn per litre of water.

Table 7.6. Chronic toxicity data for Zn, estimated by means of BLMs for rainbow trout (RT), *D. magna* (DM) and the green alga *P. subcapitata* (PS). After De Schamphelaere et al., 2003a.

Parameter	RT	DM	PS
$f_{ZnBL}^{50\%}$, %	10.4	11.7	45.4
f_{ZnBL}^{NOEC}, %	5.1	7.7	14.3*
EC_{50}, µg Zn/l	110-1970	110-370	26-1630
NOEC, µg Zn/l	32-890	48-170	4.8-610*

* for algae, the NOEC value is replaced by EC_{10}

The variations in chronic toxicity values given in Table 7.6 are based on laboratory tests with reconstituted waters covering the theoretical range of water qualities that may occur. However, the ranges of physico-chemical data actually observed in European surface waters have also been used to predict chronic toxicity to the three test organisms by means of the BLMs. When this was done using 3-year data for 411 monitoring locations in the GEMS database, representing the whole of Europe, the following results were obtained:

Range of NOEC predictions for the calculated 10th to 90th percentiles for the 50th percentiles of the 3-year GEMS data, representing the whole of Europe:

- Rainbow trout, chronic toxicity, NOEC: 120 – 1080 µg Zn/l
- *Daphnia magna*, chronic toxicity, NOEC: 110 – 760 µg Zn/l
- Green alga, chronic toxicity, NOEC: 23 – 100 µg Zn/l

It should be stressed, however, that Scandinavian surface waters are not well represented in the GEMS database. A recent compilation of water quality data from Norway, Finland and Sweden (NIVA, 2001) shows that the median values for water hardness in these countries vary in the range 4.0 – 11.5 mg/l as $CaCO_3$, the pH between 6.4 and 6.8, while the median TOC levels are 1.9 – 6.1 mg/l. The corresponding 50th percentiles in the GEMS all-European database are: 151 mg/l, 7.8 and 4.1 mg/l, respectively.

7.2 Toxicity to sediment-dwelling organisms

Bioavailability and toxicity to benthic organisms of trace metals in aquatic sediments is governed, on one hand, by the sediment properties as well as by the chemistry of pore-water and overlying water and, on the other hand, by the physiology and feeding behaviour of the benthic organisms. Lately, several studies have been conducted to test the usefulness of the SEM/AVS concept to assess the bioavailability of trace metals in sediments under more or less realistic field conditions.

In one major study, field sediments from four different aquatic habitats were investigated and then spiked with zinc (400 mg Zn/kg and 1,200 mg Zn/kg added) and returned to the original sites to allow benthic fauna to colonize the Zn-enriched sediments. It was found that the total Zn *concentration in the sediments did not show any relationship with adverse effects on benthos. No chronic toxicity was observed in sediments with SEM/AVS ratios <2. When the excess SEM was below 147 µmol/g organic carbon, no chronic toxicity was observed. On the other hand, Zn-spiked sediments with SEM/AVS ratios >8 or SEM >580 µmol/g organic carbon were found to be toxic. The authors concluded that zinc bioavailability and toxicity in freshwater sediments can be accurately predicted by the SEM/AVS model if the sediment's content of organic carbon is taken into account. However, the general applicability of this conclusion remains to be proven, because of the somewhat artificial conditions created in the sediments by spiking with great amounts of Zn and because no assessment of the vertical gradients of redox potential, AVS or dissolved sulphides from the sediment surface and downwards was made. The most plausible deviation from the accuracy of the model's predictions is that – in the real world – sediment-associated metals seem to have a lower bioavailability and toxicity than what is predicted by the model. Thus, the model seems to be a good predictor of non-toxicity of trace metals in sediments, but may not be fully reliable in predicting that a certain sediment is toxic.*

Undisturbed sediment cores from metal-polluted and reference sites were used in the second major study being reviewed. These intact sediment cores, with oxygen-rich water continuously flowing over the sediment surface, were incubated in the laboratory for several months. In this period, the change in concentrations of AVS, dissolved sulphides and trace metals was followed at different depths in the sediment core, in the pore-water, the overlying water and in the tissues of invertebrate fauna being introduced to provide bioturbation in the sediment surface. The concentration of AVS declined during incubation with oxygen-rich water, but only in the top 0–1.0 cm sediment layer. Below this level, both dissolved sulphides and AVS remained in high concentrations. Only Zn decreased in concentration in the sediment surface, but not Cu and some other trace metals forming sulphides with low solubility.

It was also demonstrated that in spite of several months of oxygenation of the originally anaerobic sediments, in which only the top cm turned aerobic, they exhibited a low toxicity in terms of mortality and embryonic malformations to the introduced amphipods. With regard to the relevance of the SEM/AVS model for predicting trace metal bioavailability to the amphipods, the authors found that the model was useful particularly for Cd, Pb and Zn, while the bioavailability of Ni, Cu and Hg was less accurately predicted, although the non-toxicity of all studied metals was well predicted. Pore-water metal concentrations had a low influence on the metal body burden of exposed amphipods, which led to the conclusion that a major route for metal uptake in the amphipods was not directly from the pore-

water, but via the food chain. However, whether the metals bioaccumulated via the food really produced any toxic responses in the amphipods could not be shown; it was rather speculated by the authors that the low toxicity of the sediment was mainly due, not to the presence of bioavailable metals but to the presence of toxic sulfides.

7.2.1 Some general considerations

Soft sediments have long been considered to be a critical compartment of aquatic ecosystems, where natural and anthropogenic chemicals accumulate. This accumulation of particulate and dissolved, organic and inorganic materials makes sediments a centre of biogeochemical cycling and a base of the food web. The benthic microbial, meiofaunal and macrofaunal communities can reflect sediment quality within a given system, which in turn reflects the quality of the watersheds that contribute to the system (Burton, 2002). Therefore, a tremendous amount of effort has been focused on how to properly assess the quality of sediments, particularly in environmental risk assessments of contaminants, such as trace metals, contained in the sediments.

The behaviour of trace metals in aquatic sediments is controlled by a complex of physical, chemical and biological factors that might transform their speciation under changing redox conditions below the sediment-water interface. Bioavailability of trace metals in sediments is determined, on one hand, by the sediment properties and by the chemistry of pore-water and overlying water and, on the other hand, by the physiology and feeding behaviour of benthic organisms (Chapman and Wang, 2001).

In oxidized sediments, iron and manganese oxyhydroxides and particulate organic carbon control the bioavailability of trace metals, while in anoxic sediments, the most important regulating factors are pH, redox potential and sulphides. In most undisturbed silty sediments there is a sharp gradient in redox potential from the sediment surface downward to deeper layers. While the sediment surface may be well oxidized, there may be no molecular oxygen present at a sediment depth of 2-5 mm (anoxic conditions), in particular in sediments rich in organic matter. Thus, for bioavailability studies with deposit-feeding invertebrates, some of which have a burrowing behaviour, it is important to consider exposure not only to trace metals at the oxidized sediment surface, but also to lower sediment layers with suboxic or anoxic conditions (Eriksson Wiklund and Sundelin, 2002).

As was discussed in detail in section 5.4.3 of this report, one of the most popular among the recent approaches to predicting metal bioavailability and toxicity in sediments has been based on the SEM/AVS concept. Lately, the significance of this tool as the most important toxicity predictor has somewhat weakened and it is now rather considered as one among several tools in the toolbox (Chapman and Wang, 2001). However, the tool seems to be more efficient in predicting non-toxicity of sediments. Since bioavailability of sediment-bound metals is not necessarily attributable only to the characteristics of sediment pore-water, bioaccumulation cannot be fully accounted for by the SEM/AVS model. In fact, some recent studies indicate that the bioavailability of sediment-bound trace metals may be more closely related to the digestive systems of certain groups of benthic organisms than to chemical extractability.

That the food route is important for trace metal uptake and toxicity in various invertebrates has also been demonstrated by, e.g., Thomann et al. (1995) who developed a steady-state model relating the ratio of metal concentrations in two species of bivalves (the oyster and the blue mussel) to sediment metal concentrations. Relevant components included sediment-water column partitioning, bioconcentration factor, depuration rate, metal assimilation efficiency from food, the bivalve feeding rate, and the growth rate of the organism. The model, calibrated with field data from the United States, indicated the food route of exposure to be significant for metals such as **zinc**, cadmium, **copper** and mercury. When LaBreche et al. (2002) used a similar model, they found for adult hard clam (*Mercenaria mercenaria*) that 94% of the **copper** intake was predicted to be accumulated via food ingestion. Of course, a key question that needs further investigation is to what extent the trace metals taken up via the food produce toxic responses to the same extent as metals taken up via the respiratory membranes.

Most of the present models that account separately for bioaccumulation via the dissolved phase versus food, however, have been developed predominantly for suspension feeders like mussels (partly for clams), which differ in feeding behaviour from the notorious deposit-feeding amphipods (e.g., *Monoporeia affinis*), which feed exclusively at the sediment-water interface. Amphipods obviously bioaccumulate trace metals from either the pore-water or the sediment phase and the extent to which bioaccumulation from the sediment phase occurs depends on both the binding strength of the metal with the sediment and the extraction capacity of gut fluids (Eriksson Wiklund and Sundelin, 2002). Again, the toxic properties of the trace metal species taken up from the sediment and extracted by the gut fluids are not yet sufficiently examined.

Nonetheless, during the last few years, a great number of studies have been conducted with the aim of testing the reliability and usefulness of the

SEM/AVS concept under more or less realistic field conditions (cf. section 5.4.3 of this report). Two of the most comprehensive among the recent efforts will be presented and commented on in the following:

- The first one used an approach consisting of spiking natural sediments from four aquatic habitats, two lakes and two riverine ecosystems (each one with low and high AVS), with zinc and then putting the spiked sediments in colonisation trays, which were returned to the original sites and allowed colonisation by benthic invertebrates for 37 weeks. The colonisation experiment was complemented with *in situ* toxicity tests.
- The second one used intact sediment cores from metal-polluted and reference sites in both freshwater and brackish-water habitats. The cores with overlying natural water were slowly oxygenated by means of a gentle flow of aerobic water over the sediment surface, which was also subjected to bioturbation by introduced amphipods. This approach allowed a long-term follow-up of the changes in vertical gradients in sediment chemistry as well as of bioavailability and toxicity of the metals originally occurring in the sediment cores.

7.2.2 Field validation of the SEM/AVS model for zinc

During the planning phase of the large-scale colonisation experiment with zinc-spiked sediments, it was clearly stated that although the AVS concept has obvious scientific merits, there are a number of questions that need to be addressed before general acceptance of this approach is justified. Among these questions, the following ones were considered pertinent to tackle within the present investigation:

- what is known about the seasonal and vertical variability in AVS in a sediment core ?
- what is known about variations in AVS between different sediment and water types ?
- is the model applicable to aerobic sediments ?
- what happens during the process of sediment oxidiation (resuspension, bioturbation) ?
- which metals, in addition to zinc, are taken into account ?
- what about dietary exposure ?
- to what extent is chronic toxicity covered by the model ?

The field validation involved aquatic habitats in four different European countries, so that different sediment and water types could be assessed during the long-term colonisation experiments and so could the seasonal variability, because samples were collected in June, September and December (Burton et al., 2003). However, since as much as a 10-cm thick layer of surficial sediments was collected from the various test sites and then mixed before spiking and return (in colonisation trays) to the original sites, it was not possible to assess any vertical gradients of, e.g., redox potential, AVS or dissolved sulphides in the sediment cores. Nor was it possible to assess the behaviour of the original, inherent zinc and other trace metals in the sediment, independently from the added zinc. Additions were made at two levels, 400 mg Zn/kg DM (low-Zn) and 1,200 mg Zn/kg DM (high-Zn).

The main results of the experiments, where chronic toxicity to the newly settled benthic communities was inferred from statistically significant differences in any of six benthic indices in the spiked versus reference sediments, were the following:

- no sediment chronic toxicity was observed below SEM/AVS ratios <2;
- sediments with organic carbon normalized excess SEM below 147 (or 100) µmol/g organic carbon (OC) were not chronically toxic;
- Zn-spiked sediments with SEM/AVS ratios >8 or SEM >580 µmol/g OC were toxic;
- sediments with SEM in the range 148-154 µmol/g OC showed variable toxicity;
- with one single exception, the overlying water did not show any acute toxicity in the *in situ* toxicity chambers;
- total Zn concentrations in the sediments showed no relationships with benthic effects.

The team of researchers responsible for the study concluded that zinc bioavailability and toxicity in freshwater sediments could be accurately predicted by the SEM/AVS model when the sediment content of OC is taken into account (Burton et al., 2003).

A few independent, recently reported, sets of data seem to support the conclusions that sediment-bound Zn usually has a very low bioavailability and toxicity to benthic organisms. When using natural, Zn-contaminated sediments in an experiment where Zn fluxes from bioturbated and unbioturbated systems were compared, it was found that bioturbation did not increase bioaccumulation of Zn in bivalves (Ciutat and Boudou, 2003). In another experiment (Lee et al., 2004) it was demonstrated that the length of the equilibration time was of great significance for the toxicity of Zn in spiked sediment bioassays. Amphipod (*Leptocheirus plumulosus*) mortality decreased significantly with increase in equilibration time, and a simultaneous decrease in dissolved Zn, suggesting a redistribution of Zn into more insoluble phases as the sediment aged.

A survey of SEM (in this case, SEM_{Zn}) and AVS data from freshwater sediments in various European countries was also conducted (Vangheluwe et al., 2003). It turned out that the only country presenting an extensive dataset (200 sediments) was Belgium, while only limited data (26 sediments) was available from other countries (mainly from the Netherlands). Based on a probabilistic analysis of existing data, it was found that – in a European context – some 72% of freshwater sediments do not have an excess of zinc over AVS, while some 28% of the sediments may have such an excess. However, if the content of OC in the sediment is also taken into account, so that the potential of the sediments to show chronic toxicity can be directly predicted, it was found that in 8% of the cases, the threshold of 100 µmol SEM_{Zn}/g OC is exceeded. In fact, the empirically determined threshold for effects on benthic communities was 147 µmol SEM_{Zn} /g OC (and not 100 µmol/g OC), which signifies that the real fraction of potentially toxic sediments (due to zinc chronic toxicity) in Europe would be somewhat smaller.

As indicated above, a certain number of results from recent studies of the bioavailability of sediment-associated trace metals have now accumulated, which tend to depict the existence of a more complex situation, indicating that the SEM/AVS/f_{OC} model, although a valuable tool for a better understanding of metal-binding mechanisms in sediments, may be an over-simplification of the full complexity of the relationships between trace metals and their toxicity to benthic fauna. In most cases, it appears that the SEM/AVS/f_{OC} model tends to provide too conservative predictions, i.e. predictons of toxic effects caused by sediment-bound trace metals are more frequent than the observed frequency in tests where both prediction and observation are conducted. Thus, there is need for continued research to refine the model and ensure that the most attractive feature of the model – its theoretical basis – can be fully exploited. However, the model appears to be quite efficient in predicting non-toxicity of metal-containing sediments. Some relevant results from recent studies were presented in section 5.4.3, and some additional findings are accounted for in the following section, 7.2.3.

7.2.3 Metal release and toxicity of sediments from the Stockholm area

7.2.3.1 Background and experimental design

A central problem in the assessment of the environmental impact of metals in metal- contaminated anoxic sediments is to estimate to what extent such sediments with high contents of organic matter will release metals once they become oxidised as a result of pollution control and/or supply of oxygen-rich bottom water. Will divalent cationic metals that were firmly bound as sulphides in the anoxic sediment be mobilised soon after oxidation of the sulphides and become bioavailable and perhaps toxic to the macrobenthic organisms that will be able to colonise the now aerobic sediment surface? Very few such experiments have been conducted, where authentic, metal-polluted sediments from lakes or estuaries, have been collected, oxygenated and then used for long-term exposure of test organisms in the laboratory, in order to distinguish toxic effects caused mainly by sulphides in the sediment from effects caused by metals (and perhaps organic toxic compounds).

In order to examine this and some associated questions pertinent to the situation in the inner water-ways around the city of Stockholm, two researchers from Stockholm University (B. Sundelin and A.-K. Eriksson Wiklund) carried out two major series of experiments. In the first series, they collected undisturbed, contaminated sediment cores from two sites located in the heavily polluted, estuarine inner part of the Stockholm archipelago, one east of Slussen at 38 m depth (stn. 1) and the second one at Svindersviken at 6 m depth (stn. 3). Sediment cores were also collected from an uncontaminated reference site, near Askö, south of Stockholm, at 37 m depth (stn. 2). Most of the undisturbed sediment cores were incubated in the laboratory for several months with a continuous flow of well oxygenated brackish water passing over the sediment surface, while some of the cores were used for metal analysis after having sliced the core in 0.5 cm thick layers. In the upper four slices, As, Cd, Cu, Ni, Pb and Zn were determined as total, dry matter-normalised metals after digestion in nitric acid according to Swedish Standard. This first round of experiments was published in 2001 (Sundelin and Eriksson, 2001).

In addition to metal analyses, determination of dry matter and organic matter (as loss on ignition, LOI) was carried out, and in each of the 0.5 cm slices down to about 10 cm sediment depth, redox potential, dissolved sulphides and AVS were analysed immediately after collection of the sediment cores and then after 3 or 7 months incubation in the laboratory with or without bio-turbation caused by addition of a number of amphipods (Monoporeia affinis). Thus, it should be noted that although the authors determined the acid-volatile sulphides (AVS) at many levels in the sediment cores, they did not determine the SEM, but chose to make analyses of total concentrations of metals instead, see Table 7.7.

Table 7.7. Total, dry weight-normalised concentrations (mg/kg dry matter) of some trace metals in the sediment surface (0-0.5 cm) at the three sites, investigated by Sundelin and Eriksson (2001) (mean value of three replicates).

Metal	Station 1	Station 2 (ref.)	Station 3
Cadmium	3.3	1.0	9.3
Chromium	100	80	106
Cobalt	31	16	15
Copper	340	40	610
Lead	340	60	990
Nickel	38	43	38
Zinc	730	190	1070

LOI in the surface sediment at stn.1 and 3 was 27.6% and 19.8%, respectively, while it was about 14% in the reference sediment from Askö.

The laboratory incubations were made in two separate experiments, in two consecutive years. The first experiment included sediments from stn.1 and 2 and lasted for 7 months, at the end of which the amount of trace metals removed from the sediment surface as a result of oxygenation by the flow-through brackish water was determined. Moreover, the accumulation of metals in the introduced amphipods over a period of 4 months was measured. Experiment 2 included sediment cores from stations 1, 2 and 3, and in addition to the metal analyses of sediment and amphipod tissue, the changes in levels of total sulphur (including AVS and dissolved sulphides) were followed, as were the toxic effects on the amphipods by observations of the frequencies of dead eggs and embryo malformations (Sundelin and Eriksson, 2001).

A second round of experiments was made at a later stage, where sediment cores from the three above mentioned sites were used in new laboratory incubations for 40 days under oxic conditions in the presence of amphipods providing bioturbation. This time, however, the experiment included also sediment cores from three sites in the freshwater Lake Mälaren, west of Stockholm, one reference site and two sites with metal-polluted sediments. Again, the main objectives were to assess the degree to which sediment-bound trace metals would be released during bioturbation and oxygenation, as well as the significance of different factors determining the bioavailability of metals to benthic fauna. The authors also wanted to examine the relative importance of porewater metals versus sediment-bound metals for explaining the metal body burden in the ampnipods (Eriksson Wiklund and Sundelin, 2002).

In this second round, not only AVS determinations (in 0.5 cm slices down to a sediment depth of 2.0 cm), and total metal analyses in porewater and bulk sediment (down to 1.0 cm depth) were made, but also SEM analyses in the top 0.5 cm layer of the sediment cores. Moreover, specimens of M. affinis were sampled after oxic incubation in the sediment cores and were allowed to empty their guts before chemical analysis of their body burden of trace metals.

7.2.3.2 Experimental results: transfer of metals from sediments to water and organisms

The temperatures corresponded to the *in situ* conditions during the experiments, and the bottom water just above the sediment surface in the flow-through units was saturated with oxygen. In both rounds of experiments and in both the freshwater and brackish-water environments, oxygenation in combination with bioturbation substantially decreased the concentrations of sulphur and AVS in the reduced sediments, obviously a result of oxidation of sulphides. It should, however, be noted that AVS only declined in the surface 0-1.0 cm sediment layer. Below this level, both dissolved sulphides and AVS remained in high concentrations. Only in the freshwater reference sediments (with low content of organic matter and sulphur), having an originally low content of AVS, there was no decline of AVS noted in the top layer during oxygenation (Eriksson Wiklund and Sundelin, 2002).

The trace metal concentrations in the bulk sediment were only slightly changed during the oxic incubation with bioturbating amphipods. Only **zinc**, forming one of the most soluble metal sulphides, decreased in concentration, especially in the brackish-water sediments.

Metals forming sulphides with lower solubility, such as **copper**, mercury and lead, did not show any change of concentration in the sediments, even in cores with high degree of bioturbation. In other studies it has been found that during oxygenation of anoxic sediments, the more soluble sulphides FeS and MnS are released first, while trace metal sulphides remain unoxidized. Possibly, FeS and MnS, which occur in excess compared to trace metal sulphides, might buffer the effects of bioturbation, leaving the trace metal sulphides unoxidized for some time (Eriksson Wiklund and Sundelin, 2002). In fact, Simpson et al. (2000) observed that PbS and CdS and even **Zn**S were resistant to oxidation, while AVS was rapidly oxidized.

When concentrations of trace metals in the porewater were followed, it turned out that, before incubation, higher concentrations of trace metals were recorded at 0-0.5 cm depth than at 0.5-1.0 cm sediment depth. After oxic incubation, however, the opposite pattern appeared: there was an increasing trend at 0.5-1.0 cm for cadmium, iron and **zinc**, metals forming the least water-insoluble sulphides among those examined.

When looking at the metal flux during oxic incubation from the sediments to the amphipods, it was observed that the bioaccumulation of **copper** and **zinc** was low, possibly due to internal amphipod regulation of these essential trace metals. The small variation in body burdens of these metals showed no correlation with metal concentration in bulk sediment or in the porewater. The body burdens of some other trace metals, such as cadmium and lead, were elevated in the amphipods. An important observation was that the concentration of these metals in the top sediment layer (0-0.5 cm), but not the concentration in the porewater, seemed to govern their concentration in the amphipods. This led the authors to conclude that food rather than the porewater would be the main source of the trace metals recorded in the *M. affinis* (Eriksson Wiklund and Sundelin, 2002).

7.2.3.3 Experimental results: toxicity to amphipods

A slight toxic effect was observed on reproduction of amphipods exposed to the oxygenated, contaminated sediments from the brackish-water area, as shown by a slight increase in frequency of undifferentiated eggs, dead eggs and general mortality, compared to the results from the control sediments. The frequency of malformed embryos did not differ between the two contaminated sediments, but was slightly increased as compared to the controls ($p<0.05$). The authors concluded that the experimental results obtained with the sediments from the two sites in the inner Stockholm archipelago indicated a *"low toxicity of the sediments"*. Moreover, they concluded that *"the higher frequencies of dead eggs observed in station 1 sediment as compared to station 3 sediment could be due to dissolved sulfides, which have been correlated with increased levels of dead eggs and broods."* (Sundelin and Eriksson, 2001).

7.2.3.4 Interpretation of results

The experiments combined four approaches to the assessment of the transformability of sulphide-bound trace metals into mobile and bioavailable species, in situations where sulphides are oxidized: (i) determining the decline in concentration of “total” trace metals and changes in SEM in the top layer of the sediment; (ii) measuring changes in concentrations of porewater metals at two different sediment depths; (iii) measuring bioaccumulation of trace metals in benthic, deposit-feeding fauna; and (iv) observing lethal and non-lethal toxic effects on the benthic organisms. As confirmatory evidence, the disappearance of AVS and dissolved sulphides in different sediment layers, as a response to the long-term oxygenation, was followed.

In the sulphide-rich sediments with very low redox potential, the divalent, cationic trace metals were originally immobilised as non-bioavailable metal sulphides. The results reported by Sundelin and Eriksson (2001) showed that oxygenation, separately or in combination with bioturbation, conducted during periods of three or seven months, was not sufficient to release some of the trace metals (those forming sulphides with particularly low solubility, such as Pb, Hg and **Cu**). However, Cd and **Zn** concentrations declined upon oxygenation in the contaminated sediments.

Based on the low AVS levels in the top layer of the sediments after oxygenation, it is difficult to explain the low level of toxicity only on the basis of AVS normalisation. According to the authors, the low bioavailability of Cd, **Cu** and **Zn** could also be explained by association with total organic carbon or re-precipitation into iron hydroxide formed as a fluffy precipitate on the sediment surface after the extended oxidation of FeS in the sediment. Since both **Cu** and **Zn** are essential trace metals, and amphipods are known to be able to regulate these metals (Borgman and Norwood, 1995), the lack of bioaccumulation could furthermore be due to the internal regulation process of the organisms (Sundelin and Eriksson, 2001).

One of the major conclusions drawn from the present experiment was that in spite of several months of oxygenation of originally anaerobic sediments, which was supposed to oxidize sulphides and mobilise toxic trace metals, the aerobic sediments showed a low toxicity in terms of mortality and embryonic malformations, to the introduced amphipods, *Monoporeia affinis* (Sundelin and Eriksson, 2001). With regard to the relevance of the SEM/AVS model for predicting trace metal bioavailability and toxicity to amphipods, the authors found that the SEM/AVS concept may be useful only for Cd, Pb and **Zn**, while the bioavailability of **Ni, Cu** and Hg could not be accurately predicted by this ratio since the ability of HCl to extract these metal sulphides is limited (cf. section 5.4.3.3 and Cooper and Morse, 1998).

Since AVS in the top sediment layer was rapidly oxidized during oxic incubation, while metal sulphides stayed stable to oxidation, the authors refer to Simpson et al. (2000), who suggested that reactive sulphides play a limited role in controlling the speciation of many trace metals entering sulphide-rich sedimentary environments. Moreover, as was also pointed out by Chapman and Wang (2001), bioavailability of sediment-bound trace metals is more closely related to the digestive systems of benthic animals. This to some extent seems to contradict the hypothesis that metal bioavailability is mainly controlled by the geochemical equilibration of metals between pore-water and reactive sulphides (Eriksson Wiklund and Sundelin, 2002).

Incidentally, a growing number of reports do emphasize the role of the food route for uptake of trace metals in benthic fauna. Chen and Mayer (1998) demonstrated that considerable fractions (10-30%) of sedimentary **Cu** can be solubilized by gut fluids, resulting in elevated Cu concentrations of 55 to 4,400 µM in the gut fluid of the deposit-feeding lugworm *Arenicola marina*. These results suggest that guts of deposit-feeders could be exposed to high concentrations of dissolved Cu (and other trace metals) during *in vivo* digestive processes. Copper can interfere with enzyme function, and the gut is a site of intensive enzyme activity. It was recently demonstrated that there is a widely differential susceptibility among benthic species to gut enzyme inhibition by Cu (Chen et al., 2002). Inhibition of digestive protease activities was found to occur at threshold values for Cu varying from 8 µM to 0.4 M among the 34 deposit or suspension feeders tested. Based on the 10th to 50th percentiles of the threshold values, and assuming that about 20% of the sediment-bound Cu is bioavailable to gut fluid solubilization, it was demonstrated that sensitivities of different benthic organisms to total Cu levels in sediments would range from 50 to 435 mg Cu/kg DM. This is a less noticed mechanism of trace metal toxicity, in addition to the previously discussed respiratory membrane-toxicity, an aspect that should be considered when assessing environmental risk of trace metals in sediment and soil habitats under oxic conditions.

Another major conclusion made by Eriksson Wiklund and Sundelin (2002) was that the observed low release of trace metals from sediments and the low impact of porewater metal concentrations for the metal body burden in exposed amphipods altogether indicated that ameliorated oxygen conditions in metal-polluted sediments may be regarded as a minor environmental threat for the important benthic organism *Monoporeia affinis*. Thus, the risk that reversal of eutrophication in the inner Stockholm archipelago would bring about another environmental risk, i.e. mobilization of sediment-bound toxic metals and transfer of metals to higher levels in the food chain, does not appear to be imminent.

This conclusion is furthermore supported by the previously cited work by Ciutat and Boudou (2003), where it was demonstrated that bioturbation of natural, Cd- and Zn-contaminated sediment, caused by nymphs of the mayfly species *Hexagenia rigida*, resulted in a significant metal release into the water column via the resuspended sediment particles. However, the use of the bivalve *Corbicula fluminea* as an indicator of uptake of metals from the water column showed that the metal bioavailability was very limited; levels of Cd and Zn bioaccumulated in the soft tissues of the bivalves being less than those measured in the unbioturbated systems.

An additional indication of the existence of a different route of entry to benthic animals of sediment-bound metals, as opposed to the « gill route » was given through the work by Milani et al. (2003), who compared the relative sensitivity of four different benthic invertebrates to metals in spiked-sediment exposures and made the same comparison with water-only exposure to the same metals. In the water-only exposure, the animal being most sensitive to Cu exposure (measured as survival and growth/reproduction) turned out to be *Chironomus riparius*, while in the spiked-sediment Cu exposure, the two most sensitive animals were *Hyalella azteca* and *Hexagenia* spp. The different order of sensitivity among invertebrates to water-borne Cu and sediment-associated Cu indicates that the spiked sediment cannot simply be regarded as a source for release of Cu to the dissolved phase.

7.2.3.5 Some remarks on experimental design in sediment research

One of the greatest difficulties in understanding the processes occurring in aquatic sediments is the problem of obtaining information from the system without disturbing it. The studies conducted by Sundelin and Eriksson (2001) and Wiklund Eriksson and Sundelin (2002) were performed with a low degree of disruption of the original sediment structure. Therefore, the presented results are supposed to be valid for *in situ* conditions in situations where originally anaerobic and metal-contaminated sediments are oxygenated and transformed into sediments having an aerobic surface layer with reasonable conditions for survival of a benthic faunal community. It should, however, be noted that the amphipods, used in the experiments to follow biotic uptake and toxicity, were artificially introduced to the sediments and, thus, were not pre-exposed to the actual metal concentrations.

Nonetheless, the use of field-collected, undisturbed sediment cores, which are brought to the laboratory for carefully controlled, long-term experiments, does contribute to a realistic set-up that provides a good basis for correct interpretations. For example, the ageing of natural sediments often results in much slower contaminant desorption rates than is experienced from unaged laboratory-spiked samples (Adams et al., 1992 and section 5.5.6). Moreover, laboratory tests with spiked sediments generally result in higher toxicity levels as compared to toxicity tests with contaminated sediments brought from the field (see e.g., Sasson-Brickson and Burton, 1991).

It is interesting to notice that even after up to seven months of oxygenation of the sediment surface, it was only in the upper 1 cm of the sediment cores where the redox potential increased radically and the dissolved sulphides disappeared, while the AVS declined, but never reached very low levels. From a sediment depth of about 2 cm and below, the redox potential remained low, and the dissolved sulphides and AVS level varied around a relatively high mean value. Thus, from this dynamics it can be inferred that the relatively low rate of metal release during the oxygenation experiments took place only from the upper 1 – 1.5 cm of the sediment cores. This was also confirmed for the metals Cd and Zn. Metals in deeper sediment layers can be assumed to stay relatively immobile for long periods of time, and in that position they may not show any bioavailability or toxicity, except – of course – if resuspension or other mechanical disturbance of the sediment takes place.

7.3 Toxicity to soil-dwelling organisms and to higher plants

In spite of a considerable amount of effort by scientists and regulators in many different countries, it is not an easy task to define clear and unequivocal soil quality guidelines for trace metals. The various ways of expressing limits for metals in soil, either as total metal in bulk soil, as metal dissolved in the soil solution or as free metal ion activity, can all be subjected to criticism, because for one reason or another, they are not equivalent to the bioavailable fraction of the metal in question. Moreover, "the bioavailable fraction" is also a variable, since it might not be the same for a higher plant, a soil invertebrate or a soil microorganism. In stead of giving numerical values specifying a kind of global limit for each trace metal in the soil, some authors suggest that it is necessary to consider the regional natural background and formulate the permissible level of metal *contamination in terms of "critical" enhancement, i.e. the permissible number of times metal concentrations can be increased relative to the background level. But again, freshly added metals have a different behaviour (bioavailability and toxicity) in the soil than metals gradually introduced over a long period of time; the problem of metal aging in the soil has to be taken into account.*

In order to overcome the difficulty of defining threshold values for metals in soils, is has been proposed to use, in stead, "critical" concentration in the tissues of plants growing on the soils to be assessed. For Cu, the critical plant tissue concentrations appears to be in the range 10–30 mg Cu/kg dry tissue, and for Zn it might be in the range 200–300 mg/kg. However, metal "hyperaccumulators" may exhibit much higher metal concentrations in their tissues.

Comprehensive studies of metal speciation, bioavailability and toxicity in urban soils with a long history of heavy contamination with mixed metals are rare. Metals in the soils at an old railway yard in Montreal were recently found to show a low solubility, and those occurring in the dissolved phase were largely bound in metal-fulvic acid complexes. Wild plants growing in the yard accumulated much less metals than the same plant species used in short-term pot experiments. Soil respiration was not affected, despite the very high metal concentrations in the bulk soil (1,000–1,600 mg/kg for Cu, Pb and Zn), but soil nitrification was inhibited at the most contaminated sites, confirming the generally high metal sensitivity of this function.

Another major study was conducted with 15 uncontaminated topsoils with total Zn levels ranging from 7 to 191 mg/kg dry matter, which – after spiking with Zn – were used in 6 different bioassays to assess the bioavailability and toxicity of Zn in these different matrixes. The ranges in no-effect concentration obtained in the 6 assays were generally larger among soils than among tests, confirming the major role of soil properties for the toxicity of Zn. Based on the relationships observed between toxicity and various soil parameters, it would be possible to normalise the toxicity data with regard to soil type, and thereby derive "soil sensitivity factors" that would explain the differences in toxicity.

A second part of the above referred study consisted of a comparison between the effects of Zn originating from gradual field contamination of soils (from galvanized transmission towers) and those of Zn in freshly spiked soils. Zinc toxicity was consistently lower in the field contaminated soils than in the corresponding spiked soils, as demonstrated in three microbial assays and one plant growth assay. This confirms again the importance of aging for the expression of soil toxicity and perhaps also that microbial *communities may adapt to long-term exposure to a trace metal in the soil, which may reduce their sensitivity.*

7.3.1 Some general considerations

Most soil and sediment quality guidelines do not distinguish among the various forms of metals, why insoluble, nonreactive and nonbioavailable forms are erroneously deemed as hazardous as highly soluble, reactive and toxic forms (see chapter 5). We do also know that soil physico-chemical characteristics influence metal bioavailability and toxicity due to interactions with metals, and a direct impact on the selected test method.

It seems reasonable that to ensure adequate environmental protection at the lowest cost, trace metal limits for soils aiming at minimising ecotoxicological risk should be based on the soil metal pool that is actually posing a threat to organisms (plants/crops, microorganisms, soil invertebrates), i.e. the bioavailable fraction. However, although large efforts have been devoted to this problem for a long period of time, scientists have still not been able to provide simple, chemical-analytical procedures by which the bioavailable fraction of trace metals in soils can be determined. Therefore, we are still left with a situation where a complicated surrogate procedure must be used to approximate the concentration of bioavailable metal. The issue is even more complicated by the fact that the fraction being bioavailable for a certain plant species may not necessarily be equivalent to what is bioavailable for a certain invertebrate, because uptake mechanisms differ between organism groups and species.

For example, for **copper**, it has been shown that the fraction dissolved in the soil solution is not equivalent with the bioavailable fraction, because most of the copper in solution is associated with various soluble inorganic and organic complexes which do not penetrate organism membranes. In some studies it has been found that >98% of the soluble copper in soil occurs in the form of organic complexes (Landner et al., 2000). It is currently widely believed that the most reliable predictor of the bioavailable amount of copper in a soil sample is the free cupric ion activity. This can be theoretically calculated, based on knowledge about the total concentration of soil copper and the various properties of the soil that influence the equilibrium concentration of free cupric ions. As an alternative, the activity of cupric ions can be measured by means of a cupric-ion-specific electrode.

The main drawback of using the free metal ion activity as a predictor of bioavailable metal in a soil is that it describes a static situation, while trace metal uptake by organisms is a dynamic process. Each biotic species can be considered as one of the soil phases next to the particle phase and the liquid phase of the soil itself. Equilibration processes are assumed to take place between all phases present, and since the equilibration period probably is relatively long for the biotic phase compared to most physico-chemical processes, the time-dependent, dynamic aspect of trace metal bioavailability must be taken into account.

In general, assessing the risk of metals is mainly based on laboratory toxicity data with various test species, from where a species sensitivity distribution (SSD) is fitted and a "hazardous concentration" (e.g. HC5, which means a concentration hazardous for 5% of test organisms) and "no effect concentration" (e.g. PNEC) for the ecosystem is derived. But terrestrial toxicity varies not only between biological species but also with soil characteristics, which control metal bioavailability. Lock and Janssen (2001) have stressed that without accounting for changes in bioavailability there is a risk for under- or overestimation, which is especially true for essential metals, like zinc and copper, where not only toxicity but also deficiency may occur (cf. section 7.4).

Lock and Janssen (2002) also critisized that current standard chronic terrestrial toxicity tests with invertebrates do not consider effects at all life stages (multi-generation toxicity), which might mean that the toxicity of the tested metal is likely to be underestimated.

It has repeatedly been found that laboratory bioassays with soil organisms yield results that indicate higher apparent toxicity of trace metals than what is found in field studies. "Critical" metal concentrations (e.g. lowest observed effect levels to soil microorganisms) have been found to be about 5 times higher (i.e. lower toxicity) when determined in field studies than when determined in laboratory bioassays (Witter, 1992). This is probably due to the fact that equilibration of the trace metal between the different phases of the soil has not been reached in the often short-term exposures being used in laboratory bioassays. The same lack of full equilibration is often observed in experiments, where authentic soils are freshly spiked with trace metals in the laboratory. Therefore, long-term field experiments or follow-up studies of soils that have been slowly contaminated in the field over long periods of time may be necessary to estimate the real "critical" concentrations or toxic thresholds of trace metals in soils.

In spite of this, most soil quality criteria are based on ecotoxicicty tests with freshly spiked soils. These quality values may overlap with background concentrations due to the fact that both background and toxicity data are given as total, not as bioavailable concentration. There is usually a lower toxicity due to aging effects resulting, e.g., in the fixation of anthropogenic metals by clay and metal oxyhydroxides. We still lack a profound knowledge about the factors and processes governing aging effects (cf. section 5.5.6).

Lock and Janssen (2001) further discuss the role of the most relevant bioavailability-modifying parameters in soils, such as adsorption phases (clay, organic matter, metal oxyhydroxides), the amount of available sorption sites (CEC), pH and competitive sorbed ions. Concerning the porewater hypothesis or the equilibrium partitioning concept, which is based on the assumption that the porewater concentration and sorption behaviour of a metal are most relevant, it is argued that porewater is relevant only when this really is the main uptake route to biota.

Recently, it has been clearly demonstrated that the time of contact between soil and trace metal is a critical factor in determining bioavailability. The longer the metal is in contact with the soil, the greater the strength of bond formed or the more stable the solid phase formed (McLaughlin, 2001). Both adsorption and precipitation reactions exhibit time-dependent reaction rates. For a metal that has been adsorbed, this increasing strength of bond may be due to a rearrangement of the metal on the surface of the solid phase, e.g. diffusion into micropores on the surface of the soil mineral or organic material. Precipitation processses are most important where metal loadings are high, or where counter ion concentrations are high, e.g. phosphate, carbonate. A second precipitation process that may lead to reduced bioavailability of metals is where new solid phases form in soil and occlude available metal, thereby removing it from the bioavailable pool. Finally, there is the possibility that metals become more tightly bound in organic materials, either through diffusion into organic molecules or through occlusion in the organic molecule. Irrespective of mechanism, the end result is that the availability of the metal ion decreases with time of contact with the soil (McLaughlin, 2001, Smolders et al., 2003).

There is an increasing body of evidence suggesting that soil microorganisms are far more sensitive to trace metal stress than soil invertebrates or plants growing on the same soils. Moreover, when expressing toxicity threshold values for soil microorganisms or soil microbial processes, it is not as evident as for phytotoxicity (or for invertebrate toxicity) that the free ion activity (or otherwise, the directly bioavailable fraction) should be the primary basis for the assessment, because microorganisms, especially the heterotrophic ones (and their consumers), may – theoretically – be exposed to various forms of adsorbed and organically complexed trace metals as well. However, empirical studies of Cu toxicity in soil usually show a good correlation between free Cu^+ activity and toxicity to sensitive microbial processes (see section 7.3.2.1).

7.3.2 Summary of toxicity thresholds for soil organisms and plants

7.3.2.1 Copper

In undisturbed soils, it appears that most of the plant copper is assimilated by root interception of ions without much movement by mass flow or diffusion in the soil solution. A Consequence of this is that copper absorption decreases markedly when root growth in the topsoil declines and stops. On the other hand, the uptake of copper by plants is enhanced by fungi (mycorrhizae) associated with the roots of certain species. Almost all of the copper in the root environment (the rhizosphere) is organically complexed by exudates from the roots or by soil humus compounds, but it is generally understood that the metal dissociates from these organic molecules before being absorbed as Cu^{2+}. The root absorption mechanism is not entirely understood, but it obviously includes a great number of steps and may be influenced by a number of external as well as internal factors (Allen, 1999).

At the normal copper concentrations in soils reported (0.3 – 250 mg Cu/kg DM), plants rarely if ever show symptoms of toxicity or of adverse growth effects. Crops are often more sensitive to copper than the native flora, so protection levels for agricultural crops, ranging from 25 mg Cu/kg DM to >100 mg/kg DM, in general seem to be adequate for plant protection in general (IPCS, 1998). However, chronic or acute effects on sensitive plant species may occur in some soil types at copper levels in the range 50-150 mg Cu/kg DM. When copper levels in soils rise above 150 mg/kg, we begin to find more and more native and agricultural species showing chronic effects.

Generally speaking it is, nonetheless, very difficult to establish a clear threshold level for copper toxicity to plants, expressed in terms of total copper concentration in the soil. It has been suggested that a possible solution to this dilemma might be to express toxicity threshold values for copper (and other trace metals) in terms of concentrations in plant tissues, preferably in young shoots, rather than as concentration in soil. The most likely "critical" copper concentration in plant tissue appears to be in the range 10 – 30 mg Cu/kg dry tissue, but more exact values have to be determined for each of the important crops and soil categories (Landner et al., 2000).

The most sensitive tests with soil invertebrates seem to justify a toxicity threshold level in the range 30 – 40 mg Cu/kg DM (as total soil copper) for this community, at least in sandy soils.

Dumestre et al. (1999) studied the effects of copper on microorganisms in relation to its speciation. They found that the soil solution free Cu^{2+} activitiy (pCu^{2+}) proved to be the best predictor of soil Cu toxicity determined as microbial respiration lag period (LP), and hence of the soil quality, and that pCu^{2+} could integrate the existing soil physicochemical variability. Although correlating well with pCu^{2+}, maximum mineralization rate was found not to be a good effect, because of its high sensitivity to other soil charcteristics, like soil organic matter (SOM).

For most practical purposes, direct determination (ion-specific electrode) or calculation of the free cupric ion activity appears to be a sufficiently good measure of the bioavailable copper fraction in a soil. Recent data support the idea that the predominant copper species being absorbed by plant roots is the free cupric ion, and good correlations have usually been obtained between the free ion activity and results of toxicity tests with soil organisms (Sauvé et al., 1998). Based on an analysis of 31 different laboratory bioassays of copper toxicity in the soil ecosystem and a recalculation of the soil total copper concentrations corresponding to free ion activities that caused 25% inhibition in the various bioassay endpoints, it was found that the effective concentration was 20 mg Cu/kg DM in soils with pH 6.0, and 140 mg Cu/kg DM in soils with pH 7.0.

The calculated toxic threshold copper concentrations for soil invertebrates, soil microbial activities or soil microorganisms , are usually based on laboratory assays, and must therefore be utilized with caution. The threshold levels cannot be directly translated to inhibitory concentrations in the field, because laboratory bioassays tend to overestimate toxicity by up to 5 times (see above). However, the data given by Sauvé et al (1998) are useful to illustrate the strong influence of pH on toxicity threshold in the soil solution (Landner et al., 2000).

An important observation in this context is the existence of a relationship between the original, background concentration of total copper in the soil and the permissible addition of copper up to the level where the “critical” concentration is reached. Thus, the “critical” number of times the total copper concentration in agricultural soils can be increased was estimated to be 4 times (Witter, 1992). The corresponding “critical” elevation of copper in the mor (surface layer) of forest soils was estimated at 3 times, i.e. the number of times the copper concentration can be enhanced compared to the background concentration before adverse effects on the soil ecosystem begin to appear (Tyler, 1992).

7.3.2.2 Zinc

The toxicity of zinc to terrestrial organisms is, or course, dependent upon its bioavailability, which in turn is determined by various factors such as the speciation of zinc and the physico-chemical characteristics of the soil. The bioavailable fraction of zinc in soil has been calculated to range from <1% to 10% of the total zinc concentration. Zinc has to be in a soluble form to be taken up by plants. In the case of zinc toxicity, zinc replaces other metals (e.g. iron and manganese) in the active centres of enzymes (e.g. hydrolases and haem enzymes) (IPCS, 2001).

Uptake of zinc in terrestrial plants is significantly increased at a low soil pH, but reduced when there is a high content of organic matter. Normal levels of zinc in most crops and pastures range from 10 mg/kg to 100 mg/kg (IPCS, 2001). Some plant species are zinc accumulators, but the extent of the accumulation in plant tissues varies with soil properties, plant organ and tissue age.

As a general rule, it has been found that plants from environments poor in zinc are characterized by low zinc concentrations, while those from zinc-enriched environments have high concentrations. The critical leaf tissue concentration of zinc at which growth is affected was found for many plant species to be between 200 and 300 mg/kg DM. However, zinc phytotoxicity in leaves can depend to a large extent on the plant species, the age of the leaf and other factors, such as exposure period and exposure concentration. Concentrations of zinc that are subtoxic or non-toxic to plants may have metabolic effects higher up the food chain. The disappearance of herbivorous insects on zinc-tolerant plants is one example of differences in species-specific tolerances. Similarly, the zinc-content of zinc-efficient plants may be insufficient for optimum performance of herbivorous animals, especially if the zinc is present in a form which is not readily bioavailable (IPCS, 2001).

Van Gestel et al. (1993) exposed earthworms (*Eisena andrei*) to zinc as zinc chloride at concentrations in dry artificial soil of 100 – 1000 mg/kg. Zinc significantly reduced reproduction at soil concentrations of 560 and 1000 mg/kg and induced the production of malformed cocoons. EC_{50} values for the effect of zinc on cocoon production and the number of juveniles per worm per week were 660 and 510 mg/kg DM soil, respectively. At the end of a 3-week recovery period, reproduction had returned to normal.

Smit et al. (2002) studied the effect of zinc on the nematode soil fauna in an experimentally contaminated outdoor field plot soil with regard to Zn speciation in soil porewater and $CaCl_2$-exchangeable Zn, as a measure of Zn-bioavailability. Comparing the observed response with benchmark concentrations (like H5 and H50 values) as derived for Zn from the ‘general species sensitivity distribution' (SSD) of soil organisms, predictions of the SSD model could be confirmed, i. e. the community NOEC was in agreement with benchmark values that should protect the integrity of the soil ecosystem.

Lock and Janssen (2001) compared a zinc-spiked artificial soil and a historically contaminated field soil to examine if chronic zinc toxicity (to the springtail *Folsomia candida*) can be predicted by a surface-response model based on soil pH, cation exchange capacity (CEC) and total zinc. However, the model could not adequately predict chronic zinc toxicity in field soils, as porewater, water- and $CaCl_2$-extractable zinc were lower than predicted by the model developed from artificial soils. The authors suggested that effects of aging on the bioavailability of zinc should be taken into account in this context (cf. section 5.5.6). Also reproduction of *F. candida* in field soils was lower than predicted by models based on porewater, water and $CaCl_2$-extractable zinc from artificial soils suggesting the existence of other bioavailable zinc (or other metal) fractions and/or a possible dietary route of uptake. For this reason, both ageing and dietary uptake should become included in effect-based risk assessment of metal-contaminated soils (see section 5.5.6).

From their experimental data, Lock and Janssen (2001) resumed that porewater, water and $CaCl_2$-extractable zinc are predictable by pH, CEC and total zinc in artificial but not in field soils, and that porewater, water and $CaCl_2$-extracted zinc – but not total zinc – predicted toxicity (reproduction) in these laboratory systems. In field soils, reproduction was lower (toxicity higher) than predicted. In contrast to the potworm *E. albidus* experiments, reproduction of *F. candida* was not influenced by soil characteristics (pH and OM), and hence, this species seems to be more appropriate when assessing the ecotoxicity of zinc in various soils.

It may be concluded that if porewater and extractable zinc concentrations were the only bioavailable fractions, zinc toxicity would be easily predictable. Indeed the authors succeeded to show that reproduction of *F. candida* was accurately predicted by PLS models ('partial least squares projection to latent structures'), which are based on those fractions in artificial soils that are independent of pH and CEC. However, this modelling approach also confirmed a lower reproduction in field soils than predicted by the surface-response model confirming again the existence of other uptake routes or bioavailable fractions.

It is once more pointed out that most terrestrial toxicity tests work with high added concentrations and short equilibrium times, which inevitably result in a high acute toxicity from artificially high porewater concentrations long before effects of dietary exposure become visible. This is, of course, obstructing the final data interpretation. However, the porewater approach still seems to be appropriate to protect organisms from acute toxicity in extremely contaminated soils, although major regulatory and scientific concern is focused on moderately contaminated soils. The need to increasingly consider these special types of ecosystems and also dietary routes of exposure is again obvious.

7.3.3 Toxicity of trace metals in contaminated urban soils

A recent study was conducted in Montreal, Québec, Canada, with the aim of estimating chemical speciation of metals in urban sites and to quantify the relative metal bioavailability using uptake in plants and microbial assays (Ge et al., 2002). Therefore, comprehensive investigations of the speciation and behaviour of cadmium **copper,** lead, **nickel** and **zinc** were made at a metal-enriched railway yard. Soil total concentrations of cadmium varied up to about 30 mg/kg DM, of copper up to about 1000 mg/kg DM, of nickel up to 125 mg/kg DM, of lead up to almost 1500 mg/kg DM, and of zinc up to 1600 mg/kg DM, while pH varied between 5.8 and 8.6, and soil organic carbon between 1 and 18%. In soil solutions, free nickel and zinc ions represented as much as 72% and 62%, respectively, of the total dissolved metals. Free metal ions were present especially in soil samples with pH <6.5, while trace metals were strongly bound by fulvic acid when pH was >8.2. Copper was strongly bound by dissolved organic matter, and metal-fulvic acid complexes represented as much as 99% of the total dissolved copper. The chemical analyses showed that, overall, much of the soil metals had a very low solubility, and in the soil solution, most dissolved copper (and lead) were complexed, suggesting a low bioavailability.

The authors noted that the metal contamination levels in the investigated soils in the railway yard were high enough to prevent reuse of the site, even for industrial purposes, according to local regulations. However, the research team did not observe any drastic biological effects. For example, they were unable to find a metal pool, such as free ions or dissolved or total soil metals, that could consistently predict metal uptake by the test plant species (potted chicory) used in the study. Although some metal accumulation was found in either leaves or roots of wild plants growing in the yard, it was clearly shown that chicory grown in the pot experiments had significantly higher bioconcentration factors for the metals than wild chicory. Data from the microbial assays showed that the soil respiration was not affected by the level of metal contamination in the yard, but soil nitrification was inhibited for the most contaminated soils (enhanced levels of **copper, nickel** and **zinc**). The higher sensitivity of nitrification over microbial respiration (of glucose) reflects the larger number of microbial species able to decompose glucose. In contrast, nitrification is much more specific and has less redundancy, so if any species involved is affected by metal contamination, the result on the ecosystem is more readily observed and has larger repercussions on proper soil functioning (Andrén and Balandreau, 1999).

As a general conclusion, Ge et al. (2002) resume that the metals present in the soil of the railway yard in Montreal seem to have a low bioavailability, because most of the bioassay results suggested a lack of dramatic effects, despite high portions of dissolved free Ni^{2+} and Zn^{2+} found in soil solution. However, results also suggested that nitrogen cycling might be affected. It was not possible to sort out which metal was primarily causing this impact on nitrification, but maximum values of total soil metal (1000 mg/kg for Cu, 1500 mg/kg for Pb and 1600 mg/kg for Zn) were indeed so high that it is not at all unexpected that some biological effect would appear in these most contaminated soils.

7.3.4 Systematic assessment of zinc toxicity in laboratory spiked soils and in gradually contaminated field soils

A major joint effort was recently completed with the aim of identifying (i) the role of soil properties on zinc toxicity in soils freshly spiked with zinc chloride in the laboratory, and (ii) reconciling zinc toxicity data between laboratory spiked soils and field zinc gradients, based on differences in zinc availability in the soils (similar efforts are presently going on also for Cu and Ni; personal communication by Katrien Delbeke, ECI, 2004) (Smolders, 2003). Zinc toxicity was tested in the laboratory with regard to:

- Toxicity to microorganisms
    - potential nitrification rate (NH_4^+ substrate supplied) - PNR
    - substrate induced respiration (using glucose as substrate) - SIR
    - respiration as decomposition of ^{14}C labelled maize residue – maize-R
- Toxicity to invertebrates
    - reproduction test with *Folsomia candida*
    - reproduction test with *Eisenia fetida*
- Toxicity to plants
    - pot trials with wheat seedlings to measure yield

Fifteen uncontaminated topsoils (ranges of pH: 3.7 – 7.5 and of organic carbon (OC): 0.4 – 23%), with total zinc concentrations ranging from 7 to 191 mg Zn/kg DM, were collected from 9 European countries. In addition, contaminated soils were sampled in three transects towards galvanized transmission towers (pylons), where the soil total zinc concentrations gradually increased in the transects from background values to elevated zinc levels near the pylon: 83 – 2100 mg Zn/kg DM, 160 – 2500 mg Zn/kg DM and 76 – 3700 mg Zn/kg DM, respectively. In all the transects, there was a small variation in pH (4.8 – 5.2; 5.2 – 6.7; and 5.7 – 6.1), while the level of OC showed a tendency to increase with the zinc concentrations, i.e. higher values near the pylons (5.6 – 12.6%; 6.6 – 11.3%; and 3.5 – 7.7%, respectively).

NOEC values obtained for the 15 spiked soils in the 6 different toxicity tests showed considerable variation. When the NOECs are expressed as “added Zn” in mg Zn/kg DM and as mg Zn/ml soil solution (added + background), the ranges over the 15 soils were as shown in Table 7.8.

Table 7.8. Ranges of “No Observed Effect Concentrations” (NOECs) for Zn in 15 spiked soils. Data obtained from 6 different soil toxicity tests. After Smolders, 2003.

Toxicity test	Added Zn, mg Zn/kg DM	Total Zn mg Zn/ml soil solution
PNR (2 soils no NOECs)	38 – 420	0.2 – 18
SIR	30 – 1400	0.1 – 250
Maize-R	38 - >1800	0.1 - >4800
Plant (wheat)	60 – 1200	0.1 – 380
F. candida, reprod.	56 – 1800	--
E. fetida, reprod.	100 – 1000	--

The ranges in NOEC (added) values were generally larger among soils (for each test, see above) than among tests (for each soil, the range usually being about 10-fold or less), which confirms that soil properties play a major role for the toxicity of zinc. The variability in threshold values expressed as total Zn in the soil is explained by the variation in CEC and pH (in the tests with invertebrates and plant) or by the zinc background values in soils (microbial processes). In fact, it has been previously demonstrated that background zinc concentrations in soil clearly affect the zinc sensitivity of soil microbial processes (McLaughlin and Smolders, 2001). The authors suggested that soil microorganisms may acclimate to indigenous concentration of zinc in soil porewater and that this acclimation affects the subsequent response to zinc added as a pollutant.

Based on the relationships observed between toxicity and the various soil parameters, it would be possible to normalise the toxicity data for every set of soil characteristics, and thereby derive "soil sensitivity factors" that would explain differences in toxicity (after spiking) due to soil type (Smolders, 2003). From the three transects of field-contaminated soils, soil samples were taken at the furthest distance from the pylon and were spiked with zinc chloride to a range of total zinc concentrations similar to those in the transect. The field transect soils, contaminated with zinc over time by slow corrosion of the galvanized pylons, showed no clear evidence of zinc toxicity in any of the three microbial assays and in the plant growth assay, with one exception (plant test in one transect). In contrast, spiking these soils always yielded significant dose-response curves and total zinc EC_{50} values were on average 2.1-fold (in some case, 7.7-fold) lower than the largest total zinc in the transect. Thus, zinc toxicity was consistently lower in the field contaminated soils than in the corresponding spiked soils (Smolders et al., 2003).

An increasing use of various modelling approches to predicting the bioavailability of zinc (and copper) in soils can be noticed recently. For example, partitioning models as well as competitive adsorption models have been found to be quite promising tools to sort out those soil factors that are relevant for determining the behaviour and, ultimately, the bioavailability of trace metals in the soil ecosystem (Impellitteri et al., 2003).

7.4 Essentiality, regulation and deficiency

For essential metals, each living species has an optimal range of concentrations required for normal metabolic functioning. This "optimal concentration range for essential elements" is determined by the natural, background (bioavailable) concentrations of the metal in the species' habitat and the homeostatic capacity of the species. "Homeostasis" describes the phenomenon that organisms try to maintain their activities by controlling the concentrations of essential (and non-essential) elements inside their tissues. However, the homeostatic capacity of an organism has limits, so that when the external concentrations of an essential metal becomes too high or too low, regulation will fail and toxicity or deficiency, respectively, will result.

Organisms can also try to resist toxicity caused by trace metals by mechanisms known as acclimation, increased tolerance or genetic adaptation to increased metal exposure in the environment. In this sub-chapter, a few examples of copper and zinc essentiality and tolerance are *given. These examples are taken from the official World Health Organization documents "Environmental Health Criteria" for copper and zinc, which were issued in the framework of the "International Programme on Chemical Safety" (IPCS).*

7.4.1 Some useful definitions

It is obvious to anyone that life has evolved in the presence of metals, some of which – the essential trace metals – have become incorporated into metabolic processes crucial to the survival, growth and reproduction of organisms. As a consequence, organisms have developed various mechanisms for the uptake and excretion, regulation and detoxification of essential metals, mechanisms which – in many cases – are functioning also for non-essential metals. In their summary statement on the "Essentiality of Metals", Janssen and Muyssen (2001) point out that an element is considered essential when:

- it is consistently present in all healthy living tissues within a biological family, whereby tissue concentrations from species to species should not vary widely;
- deficiency symptoms are noted as a result of depletion or removal of the element, and dissapear when the element is returned to the tissue; and
- the deficiency symptoms are attributed to a distinct biochemical effect at the molecular level.

For essential metals, each species has an optimal range of concentrations required for normal metabolic functioning. This "Optimal Concentration Range for Essential Elements" (OCEE), termed by Van Assche et al. (1997), is determined by both the natural (bioavailable) concentrations of the essential metal in the species' habitat and the homeostatic capacity of the species, allowing it to regulate its internal metal concentration to an optimal level. Homeostasis is a concept describing the phenomenon that organisms try to maintain their activities by controlling the concentrations of essential and non-essential elements inside their tissues (see, e.g., McGrath, 2001).

An organism's homeostatic capacity has limits, however, and when the external concentrations of an essential metal becomes too high or too low, regulation will fail, and toxicity or deficiency, respectively, will occur (Janssen and Muyssen, 2001). Organisms have developed a variety of homeostatic control mechanisms to regulate internal metal concentrations, which may vary considerably between species or groups of species. Brix and DeForest (2000) listed the following main categories of regulation:

- active regulation, where stable tissue concentrations are maintained by reduced uptake or by the excretion of metal at rates similar to the intake rate;
- storage, i.e. binding of metals in various types of complexes for long-term storage in a detoxified form;
- a combination of both.

Simultaneously with the organism's own control mechanisms, the environment also contributes to the apparent "no-change" internal metal concentration through physical and chemical processes known as "buffering" (McGrath, 2001). As the concentration of metals increase, especially in soils and sediments, various solids or dissolved materials bind the metals chemically or even physically inside their structure.

Organisms can also try to resist toxicity by mechanisms known as **acclimation,** increased t**olerance** or genetic **adaptation** to increased metal exposure in the environment. These concepts are defined below, according to McGrath (2001):

Acclimation is a non-heritable trait and is seen as the response of an individual to stress. Even without genetic change, an individual (or even a population) may be resistant enough to continue to function normally upon increased metal exposure, a phenomenon referred to as "phenotypic plasticity". This type of non-genetic resistance can easily be lost when the exposure to metals decreases again.

Tolerance is an organism's ability to maintain homeostasis when exposed to a particular array of environmental factors.

Adaptation is a special kind of (metal) tolerance, meaning that the traits have evolved through natural selection in response to high metal exposure in the environment and can therefore be passed on through the genes to subsequent generations. Genetic tolerance or adaptation cannot be lost in the individual. However, it may render the individual less fit to survive in non-contaminated environments.

Some examples of acclimation and adaptation are given by Janssen and Muyssen (2001):

It has been demonstrated that test organisms (e.g., water fleas) cultured in media with low essential metal concentrations (such as Cu and Zn) exhibit an overall decreased fitness (cf. Caffrey and Keating, 1997). Moreover, organisms cultured at these low metal concentrations acclimate to these conditions and become more sensitive to stress, including metal exposure (Muyssen and Janssen, 2001). Conversely, organisms cultured in media with elevated metal concentrations may become less sensitive. Considering that laboratory toxicity test data are used for derivation of water quality criteria and the establishment of a PNEC (Predicted-No-Effect-Concentration) in risk assessments, these acclimation-induced sensitivity shifts may affect the ecological relevance and effectiveness of the test procedures. It has been stressed several times in this report that metals exhibit naturally varying background concentrations in different habitat types and depending on the metal background concentration, biological communities in these different systems may have adapted differently to the natural presence of metal concentrations, resulting in varying community sensitivities.

Rainbow (2002) reviewed the current literature with regard to metal accumulation in aquatic invertebrates. Aquatic invertebrates take up and accumulate metals whether essential or not, resulting in a great variability of subsequent body concentrations across metals and taxa. Accumulated metals may refer to metabolically available and stored detoxified metals, whereas toxicity is related to a threshold concentration of a metabolically available metal and not to the total accumulated metal.

7.4.2 Examples of copper essentiality and copper tolerance

The essentiality of copper to living organisms was recognized relatively late. It was not until 1928 when Hart et al. (cited in IPCS, 1998) showed that copper was needed for the formation of red blood cells in rats fed a milk-based diet. The anaemia in the rats could be corrected by the addition to the diet of ash from animal or vegetable sources. It was later demonstrated that the hydrogen sulfide precipitate from the ash, containing copper sulfide, was responsible for the recovery. Copper is also essential for the utilization of iron in the formation of haemoglobin.

The reason for copper's essentiality is its specific incorporation into a large number of enzymatic and structural proteins. Copper plays an important role in oxidation/reduction enzyme activities, and this is a consequence of its ability to function as an electron transfer intermediate. Thus, copper is a constituent of enzymes involved in, for example, cellular respiration, free radical defence, neurotransmitter function, connective tissue biosynthesis, and cellular iron metabolism. Copper also plays an essential role in the activation and repression of gene transcription and has a lot of additional function in the cell (IPCS, 1998).

In the "Environmental Health Criteria" for copper (IPCS, 1998), it is clearly emphasized that the above-mentioned aspects must be taken into consideration when assessing the impact of copper on biological systems. In order to illustrate the complexity of the role of essential metals in natural ecosystems, the following points may be cited with regard to Cu from the IPCS document:

> *"The adverse effects of copper must be balanced against its essentiality. Copper is an essential element for all biota, and care must be taken to ensure that copper nutritional needs of organisms are met. At least 12 major proteins require copper as an integral part of their structure. It is essential for the utilization of iron in the formulation of haemoglobin, and most crustaceans and molluscs possess the copper-containing haemocyanin as their main oxygen-carrying blood protein. In plants copper is a component of several enzymes involved in carbohydrate, nitrogen and cell wall metabolism."*

> *"Because copper is an essential element, procedures to prevent toxic levels of copper should not incorporate safety factors that result in recommended concentrations being below natural levels."*

> *"Tolerance to copper has been demonstrated in the environment for phytoplankton, aquatic and terrestrial invertebrates, fish and terrestrial plants. Tolerance mechanisms which have been proposed in plants include binding of metal to cell wall material, presence of metal-tolerant enzymes, complex formation with organic acids with subsequent removal to the vacuole, and binding to specialized thiol-rich proteins or phytochelatins."*

> *"Wood (1983) found that naturally occurring marine phytoplankton populations show a tolerance to added cupric ions which far exceeds the physiological limits of phytoplankton cultures grown in chemically defined media. The tolerance appears to be due to regulation of bioavailability of added copper by an abundance of copper-complexing agents. Coastal phytoplankton were less sensitive than continental shelf or oceanic communities. The toxicity of copper correlated more with copper-complexing capacity than with biotic species composition or community structure."*

> *"Copper-rich granules have been reported to occur in a wide variety of invertebrates inhabiting copper-polluted habitats.. . . In addition, copper deposits also appear in (the tissues of bladder-wrack (Fucus sp.) and in copper-tolerant isolates of the green alga Scenedesmus. It was concluded) that the occurrence of these inclusions could be regarded as a detoxifying mechanism because they were absent in the non-tolerant strains."*

7.4.3 Examples of zinc essentiality and zinc tolerance

As for copper, it is also emphasized in the "Environmental Health Criteria" for zinc (IPCS, 2001) that essentiality and tolerance must be taken into consideration when assessing the impact of zinc on biological systems. The following examples again taken from the IPCS document may reflect the critical role of essentiality for the bioavailability of Zn:

"Zinc is an essential element in the environment. The possibility exists both for a deficiency and for an excess of this metal. For this reason it is important that regulatory criteria for zinc, while protecting against toxicity, are not set so low as to drive zinc levels into the deficiency area."

"Zinc is important in membrane stability, in over 300 enzymes, and in the metabolism of proteins and nucleic acids. The adverse effects of zinc must be balanced against its essentiality. Zinc deficiency has been reported in a wide variety of cultivated plants and animals, with severe effects on all stages of reproduction, growth and tissue proliferation. Zinc deficiencies in various crops have resulted in large crop losses worldwide. Zinc deficiency is rare in aquatic organisms in the environment, but can be induced under experimental conditions."

"Zinc tolerances in terrestrial plants, algae, microorganisms and invertebrates have developed in the vicinity of areas with elevated zinc concentrations."

The main causes of zinc deficiency in crops are : (1) low total zinc concentrations in soils (in sandy, calcareous and sodic soils), (2) low zinc availability (high pH, calcareous soils), and (3) high levels of phosphate and nitrogen and restricted root zones due to soil compaction or high water table. In many parts of the world soils have been treated with zinc amendments, which has resulted in a declining trend in the occurrence of zinc deficiency. However, high yielding crops (e.g. wheat) will have a greater zinc requirement that those grown less intensively (Alloway, 2002). In a Food and Agriculture Organization (FAO)-sponsored study in 15 countries around the world, it was found that zinc deficiency is soils was the most commonly occurring micronutrient deficiency problem. Zinc deficiency was recorded in 49% of the trials and 25% of these were acute forms with visible symptoms on crops (Sillanpää, 1990).

Several important food crops can be seriously affected by zinc deficiency with maize and rice being the most sensitive crops, while wheat is moderately sensitive. Both animals and humans have critical zinc requirements, which means that in areas where zinc deficiency in crops is widespread, there may also be a high risk of detrimental effects on the health of livestock and people. The areas with highest risk of zinc deficiency in crops are situated in India, Pakistan, some parts of Africa, northwestern part of Sout America and Mexico, while Europe is a relatively low-risk area (Alloway, 2002).

As a matter of fact, the World Health Organization (WHO) has recently (2002) pointed out zinc deficiency as one of the most important risk factors to cause impairment of human health. When calculating the leading risk factors, as causes of disease burden among people in developing countries, zinc deficiency came up as number 5, while the rank of zinc deficiency is number 11 when the global situation is considered. WHO (2002) concludes that zinc supplementation and fortification of the food may be recommended in all regions of the world.

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Abbreviations

AAS	Atomic Absorption Spectrometry
ACC	Acid Consuming Capacity
ACR	Acute-Chronic Ratio
AE	Assimilation Efficiency
AES	Atomic Emission Spectrometry
APP	Acid Producing Potential
ASV	Anodic Stripping Voltametry
AVS	Acid Volatile Sulphide
BAF	Bioaccummulation Factor
BCR	EC Bureau for Chemical Reference Materials
BET-method	method to assess the specific surface area of solids by nitrogen absorption
BIOMET	Bacterial Metal-lux Biosensor
BLM	Biotic Ligand Model
C&D	Construction and Demolition Waste
CA	Component Additivity approach
CBC	Copper Binding Compound (co-factor protein)
CCM	Capacitance Model
C_E	effective concentration
CEC	Cation Exchange Capacity
CEN TC	European Committee for Standardization Technical Committee
CRM	Certified Reference Material
CSV	Cathodic Stripping Voltametry
DDL	Diffused Doubler-Layer model
DGT	Diffusive Gradients in Thin Films method
DIN	Deutsche IndustrieNorm
DLM	Diffusive Layer Model
DM	Dry Matter
DOC	Dissolved Organic Carbon
DOM	Dossolved Organic Matter
DPV/SV	Differential Pulse Voltametry/Stripping Voltametry
DS	Dry Substance

DTMP	Degree of Trace Metal Pyritization
DTPA	DiethyleneTriaminePentaAcetic acid
dw	dry weight
EC	European Commission
EC50	Effect Concentration to 50% of organisms
EDS	Energy Dispersive Spectroscopy
EDTA	EthyleneDiaminetetraAcetic acid
EDXA	Energy Dispersive X-ray Analysis
E_h	redox potential
ELV	End-of-Life Vehicles
EM	Electrophoretic Mobility
EqP	Equilibrium Partitioning
ERM	Effective Range Mean Concentration
ESR	Electron Spin Resonance
EU	European Union
EXAFS	Extended X-ray Absorption Fine Structure analysis
FIAM	Free Ion Activity Model
f_{OC}	Organic Carbon fraction in the sediment
GC	Generalized Composite approach
GEMS	Guidelines for Environmental Monitoring Systems
GSIM	Gill Surface Interaction Model
HC50	Hazardous Concentration to 50% of organisms
HDMT	Helmke-Donnan Membrane Technique
HMW	High Molecular Weight
HPLC	High Performance Liquid Chromatography
HW	Hazardous Waste
ICA	International Copper Association
ICMM	International Council on Mining and Metals
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
ICSG	International Copper Study Group
IPCS	International Programme on Chemical Safety
IRB	Iron Reducing Bacteria
ISE	Ion-Selective Electrode

IW	Industrial Waste
IW	Interstitial Water
IWTU	Interstitial Water Toxic Unit
kBq	kilo Bequerel (measure of radioactivity)
k_d	distribution coefficient
k_{OC}	Organic Carbon based partition coefficient
kt	kilo (thousand) tons
KTH	Royal Technical University, Stockholm (Kungliga Tekniska Högskola)
LA_{50}	Lethal Activity to 50% of organisms
LBRL	Lowest Biological Risk Level
LC50	Lethal Concentration to 50% of organisms
LOEC	Lowest-Observed-Effect Concencentration
LOI	Loss On Ignition
Me^{n+}	free metal ion concentration
MFA	Material Flow Analysis
MFG	MiljöforskarGruppen
MINTEQA2 and MINTEQ	Water-Ligand Geochemical Computer Models
MITF	Metal Information Task Force
MSW	Municipal Solid Waste
Mt	Million tons
MW	Molecular Weight
NEM	Non-Electrostatic Model
NGO	Non-Governmental Organisation
NOEC	No-Observed-Effect Concentration
NOM	Natural Organic Matter
NPK	Nitrogen-Phosphorus-Pottasium mineral fertilizer
OC	Organic Carbon
OCEE	Optimal Concentration Range for Essential Elements
OECD	Organisation for Economic Cooperation and Development
OM	Organic Matter
PAH	PolyAromated Hydrocarbons
PCA	Principal Component Analysis
PCB	PolyCyclic Biphenolic Compounds
PDI	Predicted Daily Intake

PEC	Predicted Environmental Concentration
pH_{IEP}	isoelectric point
pH_{PZC}	point-of-zero-charge
pK	dissociation constant
PLS	Partial Least Squares projection
PNEC	Predicted No-Effect Concentration
POM	Particulate Organic Matter
PW	Pore Water
ROS	Refractory Organic Substances
RT	Residence Time
SC	Surface Complexation
SCDA	Scandinavian Copper Development Association
SEC	Size Exclusion Chromatography
SEK	Swedish Crowns
SEM	Scanning Electron Microscopy
SEM	Simultaneously Extracted Metals
SEP	Sequential Extraction Procedure
SEPA	Swedish Environmental Protection Agency
SFA	Substance Flow Analysis
SGU	Swedish Geological Survey
SIMS	Selective Ion Mass Spectroscopy
SMAV	Species Mean Acute Value (geometric mean of LC_{50} values for a given species)
SOFIE	Sediment Or Fauna Incubation Experimental systems
SOM	Soil Organic Matter
SQC	Sediment Quality Criteria
SQG	Sediment Quality Guideline
SQV	Sediment Quality Value
SRB	Sulphate Reducing Bacteria
SS	Sewage Sludge
SSD	Species Sensitivity Distribution
STAF-Europe	Stocks and Flows Europe
STP	Sewage Treatment Plant
SWAMP	Sediment Water Algorithm for Metal Partitioning
Tc	soil-plant transfer coefficient
TDI	Tolerable Daily Intake

TEM	Transmission Electron Microscopy
TI	Toxicity Index
TLM	Triple Layer Model
TOC	Total Organic Carbon
TRS	Total Reducible Sulphide
TSS	Total Suspended Solids
TU	Toxic Unit (1 TU = 100/EC50)
UNESCO	United Nations Educational Programme
USD	US Dollar
USEPA	US Environmental Protection Agency
WEEE	Waste from Electrical and Electronic Equipment
WHAM	Windermere Humic-Aqueous Model
WHAM-SCAMP	geochemical speciation code
WHO	World Health Organisation
XANES	X-ray Absorption Near-Edge Structure analysis
XAS	X-ray Absorption Spectrometry

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