TASKS FOR VEGETATION SCIENCE – 45

Chemical Elements in Plants and Soil: Parameters Controlling Essentiality

Stefan Fränzle

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To the memory of my father Prof.Otto Fränzle (December 9th, 1932 - August 20th, 2009), distinguished natural scientist, who did not live to see this book in print but whose spirit is felt on every single page

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Chapter 1
The Biological System of Elements

By the Biological System of Elements, originally (Markert 1996) an array of chemical elements was denoted describing their distribution in green plants and abundance correlation among plant species, irrespective of biochemical "roles" (e.g., essentiality as a component of enzymes) and functions. Later on, the present author went to give a causal account which draws on different physicochemical features and necessities of biochemistry – including effects of elements other than C, N, S, O, H and P – and extending the scope of interest to other living beings and their interactions in ecosystems, that is, to stoichiometric ecology.

1.1 Principles of Element Distribution in Plants

In the beginning of the nineteenth century, analytics of plant matter samples started with that of plant ashes. In addition, no methods were available then which could have enabled intact biological materials to be digested for complete, no-loss analyses without burning them before. Hence, volatile elements then could not be detected, let alone quantified in biomass. Elements then found in plant ashes (Fe, Na, K, Ca, etc.) were both abundant and had been discovered in other sources before. As, e.g., no spectroscopic methods whatsoever were at hand earlier than about 1860, technical prospects for trace analysis then were dim at best (there are very few instances of elements detected in environmental samples/spectra prior to their isolation on Earth: helium (in 1868) and technetium (in 1952) were found in stellar spectra before being isolated from or detected in terrestrial minerals rather than synthetized by nuclear methods (Kenna and Kuroda 1962; Kuroda 1998), a third couple of emission lines (first attributed to some postulated new element "nebulium") turned out to be due to a forbidden low-pressure emission line of oxygen atoms). Amounts usually present in plants (or animals) could not be detected or measured for most elements, a problem which could be overcome only during the last decades. Now, however, advanced mass spectrometers (ICP-MS) and similarly sensitive analytic gear provide detection and determination ($<<$ 1 μg/kg DM) limits low enough to find and quantify most elements.

1.1.1 Distribution Patterns of Chemical Elements in Plants

As a rule, differences in (elemental) chemical compositions which exist among different species of (e.g.) plants should be caused by some unlike behaviour/differing processes in uptake or transport. For instance, there may be either active or passive transport of metal ions or other speciation forms of elements (complexes, oxoanions, organoelement species such as kakodylic [dimethylarsinic] acid or methylmetal [M, e.g., Hg, Pb, Tl] compounds/ions), producing different rates and/or equilibria of uptake. In turn abundance correlations among these very plant species appear which are at odds with chemical intuition, that is, a very low, virtually nonexistent abundance correlation in pairs of closely elements one of which is resorbed and shuttled onward to leaves/needles and fruits/seeds in a constant manner whereas the other is transported by ways/carriers

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which depend on the corresponding species while conversely chemically apparently unrelated elements may follow similar paths. Geochemistry, including pH and wetness of soil substrate, thus provides very different patterns of elemental abundances for metals as well as certain non-metals. There are both synergistic and antagonistic relationships between uptake or use of different elements by plants. Of course these latter interactions, which partially represent the response of the plant to local geochemical conditions, in turn change the distribution patterns by mainly antagonistic interactions among essential elements (Kaim and Schwederski 1993); also consider Fig. 1.1:

Negative abundance correlations (e.g., Ca/Mg) may indicate a direct competition for the same binding centers owing to some chemical similarity among the pair of metals (Fig. 1.3). Thus, chemical similarity can bring about both highly positive and highly negative abundance correlations depending on dynamic features: if retention to biomass dominates in the end, similar coordination properties – both concerning binding strength and ligand selectivities – will result in positive abundance correlation whereas control by transport mechanisms, including competition for low-concentration carriers, rather gives a negative correlation. However, it is unlikely that both effects will cancel, producing no discernible abundance relationship across various plant species whatsoever. Notably, Fig. 1.3 does not display dynamic features like a rate of plant growth but "simply" the abundance relationship among the elements and plant species. Thus, Figs. 1.1 and 1.2 cannot be directly compared even for identical pairs of elements.

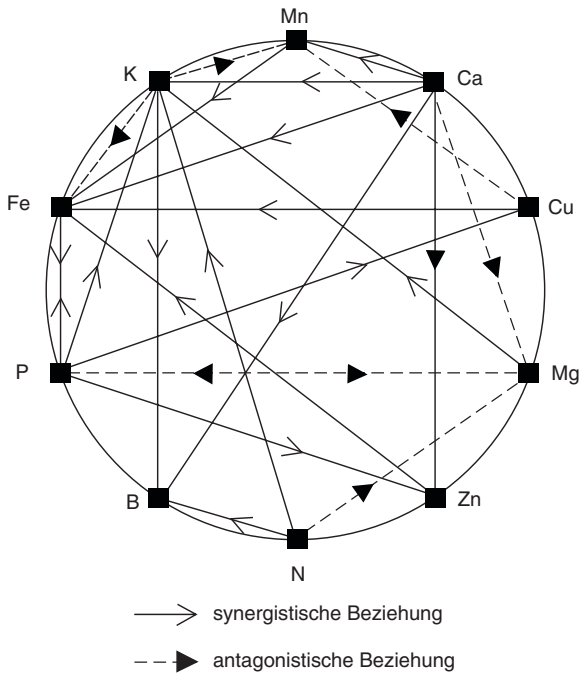

Fig. 1.1 Network of interactions/influences among some essential chemical elements in plants (Kaim and Schwederski 1993). The parameter used for attribution of either synergy or antagonisms to an interelement relationship is rate of plant growth

Local enrichment of certain elements within some plant may be due to both complexation with polymeric components of biomass and to precipitation of solid, insoluble, sometimes even crystallinic phases. Before an element may be enriched or separated in any of these kinds, three other factors contribute to the series of events, besides the conditions of uptake, namely:

- Speciation of elements next to its rhizosphere, respectively
- Mechanisms and kinetics of uptake by roots (or fungal mycelia) or leaves (especially in aquatic plants)
- Mobility inside the plant, controlled, e.g., by phosphate in the xylem

For example, the relatively large amounts of Rb present in plants may be involved in chemical signalling much like Na or K and will obviously contribute to osmoregulation, but the latter effect does not render Rb essential because it can be replaced by other ions (or even organic compounds such as glycerine) for this purpose, and other, (more) specific uses are not obvious from analysis alone. Although some chemical details of paleobiochemistry may be inferred from appropriate fossile samples such as chitin in amber inclusions, analytical data will never reveal what element actually was required by some extinct organism. Though differences in essentiality patterns among protist, animals, plants or fungi are well-known for now (Table 2.1), and "genetic clocking" allows for temporal reconstruction of the separations of their common ancestors (Feng et al. 1997), the corresponding changes among essentiality patterns upon evolutionary radiation are not accessible. This holds the more for results of thorough geochemical changes during evolution or for such extinct organisms which apparently do not fit into patterns and categories of recent-time

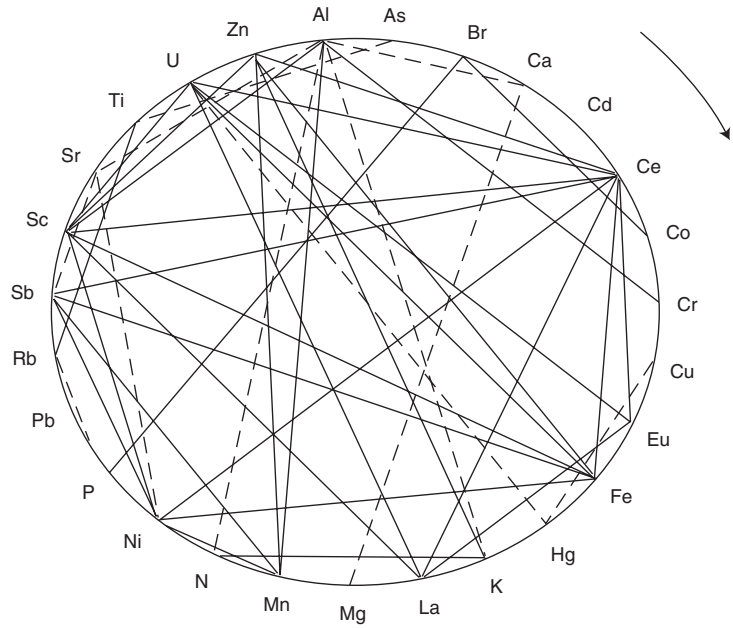

Fig. 1.2 Highly positive (*straight connection lines*) and highly negative (*broken connection lines*) abundance correlations among pairs of chemical elements in 13 species of plants (from Markert 1996)

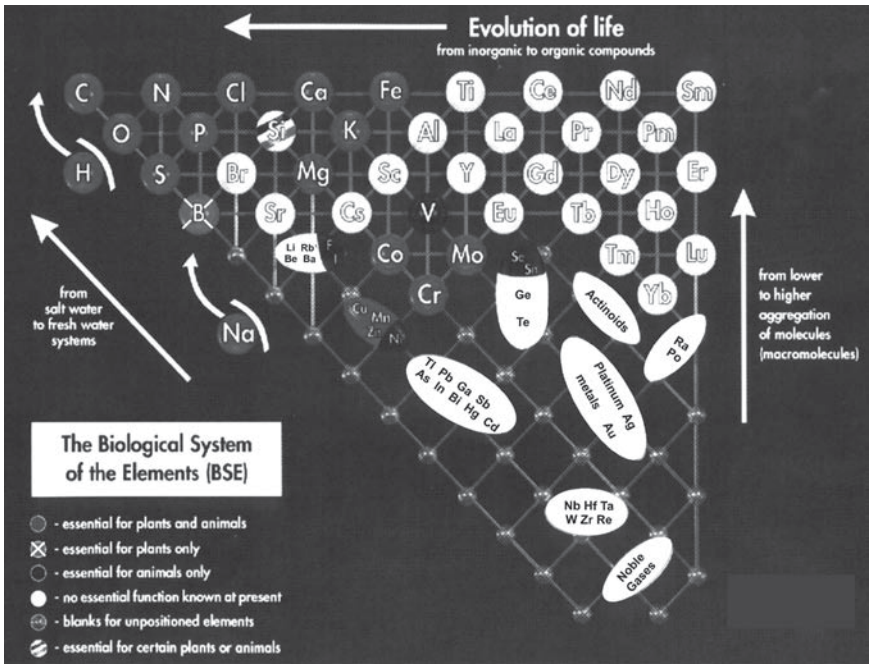

Fig. 1.3 The Biological System of Elements (Markert 1994a). The diagram shows relationships among the elements together with their corresponding essential functions (*colours*), extent of biochemical functions and the corresponding capacity to form macromolecules by condensation reactions (*vertical arrow at right side of diagram*). Whereas in "pure" geochemistry oxophilic metals produce the most complicated condensation products, i.e., clay minerals, there was a shift towards non-metal-based structures during chemical and biological evolution which afforded polymeric structures based on the latter (C, N, O) (*horizontal arrow to the left*). The diagonal arrow refers to changes of concentration from ocean- to freshwater. There is substantial decrease of concentrations in some elements (Mg, Sr, Cl, Br) from ocean to freshwater requiring them to be enriched by biomasses if their biochemical use is to be continued. Such kinds of enrichment can only be accomplished by means of certain biochemical features which involve properties and/or components of the corresponding biogenic materials – many of which are specific for one species at least in their particular combination

taxonomy at all (e.g., Venda organisms from the uppermost Precambrian).

Once, however, biocatalytic essentiality or chemical signalling with no chance to replace the components by other elements have been identified, matters change radically. Now, life is about reproducing some very complicated chemical gear – including accumulation and appropriate speciation of all the elements which are essential to that species – in an autocatalytic manner. From this point of view, a green plant is a device which produces and employs carbon compounds to obtain more C and increase its throughput/fixation rates of C (and all other essential elements) by (usually) identical reproduction of first the corresponding reactants (rubisco, chlorophyll and all the other proteins involved) and then that of metastructures (cells, entire organisms). Growth and reproduction thus correspond to *autocatalysis*.

The further line of argument is focussed on land plants for reasons of comprehensive data sets available here, yet there also are data for limnetic plants, as well as bacteria and animals. In terrestrial plants, there is a well-defined pathway for uptake and transport of metal ions rather than the chance to establish an equilibrium of element concentrations between water and biomass making use of almost the entire interface like in aquatic plants: for terrestrial plants, few, mainly epiphytes (Strasburger and Sitte 1991), are capable of taking up salts by leaves, otherwise it takes place by way of roots only. Although some (essential) non-metals such as sulfur (as SO_2) and nitrogen (both [either] as NH_3 or various nitrogen oxides) can be absorbed, used and metabolically transformed within the leaves, this pathway can be neglected for metals and so-called semimetals in higher plants and geochemically realistic conditions. In mosses, there are "colloquial" amounts of essential metals, say 35 μg/g DM of Zn and 3 μg/g DM of Cu even if there is no atmospheric deposition (extrapolation to zero). This points to a similar way of uptake to meet essential demands between mosses and vascular plants even though there are no roots in the former. Mosses give away amino acids and peptides when exposed to drought stress, causing weathering of underlying material with the apparent result of complexes to be resorbed by the mosses quite efficiently.

Speciation, uptake and transport alike depend on chemical properties and possible chemical and biochemical transformations of the corresponding element. Most of the essential non-metals (N, B, P, S, in addition Mo) are absorbed as oxoanions in their highest oxidation states whereas Cl is used as an elemental anion, that is, as Cl^-. Yet, it must be noted that some 40 – 50 % of absorbed nitrate N are converted into amino acids in the microroots already, as are – sometimes even larger – shares of other metal or non-metal oxoanions such as CrO_4^{2-}. In higher plants, substantial uptake of metals or semimetals *by leaves* would occur only if there are fairly persistent volatile forms which could be admitted to the stomata, except or particles within hydrometeors small enough to pass the stomata (or soluble in water, e.g., sea salt spray). However, permethyl compounds of Hg, As or Se have tropospheric lifetimes of minutes rather than hours given their reactivities towards OH radicals, much like peralkyls of Sn, Pb or Mo, W hexacarbonyls which are released from anaerobic layers of domestic dump pits (Feldmann 1999).

1.1.2 Biochemical Essentiality of Elements in the Light of Enzymatic Reactions

It goes without saying that occurrence of such relationships among elements does not depend on their essential functions, either of one of the involved elements or of both (for all the investigated kinds of organisms?) or even of neither. Rather, non-essential elements are likely to reveal the effects of chemical binding to plant biomass even more clearly because the influences by element-specific regulation or transport mechanisms should be less pronounced than with either essential or highly toxic elements (cp. the role of chaperons in sequestration, transport and elimination of Cu, Ni, Zn [essent.] and As, Cd, Pb [toxic] in both several plants [Tottey et al. 2005; Vernay et al. 2006] and kinds of bacteria). Farago (1986) and Clemens et al. (2002) give a very detailed picture of the processes which occur during binding of chemical elements in plants. Farago's work deals with the responses of different plants – including metal ion hyperaccumulators – towards variations of soil metal contents, focussing on morphology and mode of function of roots influencing resorption kinetics. In Farago's list, there are six dominant non-metals and besides these three macronutrients (K, Mg and Ca) and a larger number of "essential micronutrients" (Fe, Cu, Mn, Zn, Mo, Co, V, Na, Rb, B, Si, Cl, I, Se) while other elements (Ni, Al, Sr, Sn, Cr, Br and F) are considered "beneficial or of restricted essentiality" (there is some disagreement with this list, for example Ni is known to be a component of plant

enzymes including urease controlling the nitrogen cycle while Al [although accumulated by some plants like certain ferns and black tea; Kaim and Schwederski 1993;Markert 1996] and Cr are counted among plant toxins otherwise. The actual role of Rb is uncertain up to this day).

Concerning a high tolerance towards Cu, high concentrations of proline (a proteinogenic amino acid) in roots of Cu-adapted populations of the bog-plant *Armeria maritima* are presumed to be involved. Different authors (Still and Williams 1980; Farago 1986) agree that the effective fractionation between Co and Ni in *Hybanthus floribundus* may not be effected by the hydroxycarboxylic acids produced by their roots only; in addition, most of Ni in the leaves is water-soluble (-extractable) hence probably in a low-molecular state of binding. These examples – which could be amply extended – already give proof that coordination chemistry is most important for understanding the processes occurring with metallic elements in the biota. But we shall soon notice that this is not the complete story.

In the 1840s, Liebig laid the fundaments of *Agrikulturchemie* (agricultural chemistry), dealing with the question which amounts of which chemical elements are necessary to grow and maintain higher (terrestrial) plants. In 1860, iron became the first trace element established to be essential for higher plants, to be followed by essentiality determinations for Mn, B, Zn and Cu between 1922 and 1931; lateron Mo and Cl were added to this list (Marschner 1986). In 1939, Arnon and Stout coined the term mineral nutrient. According to other investigations some 13 chemical elements, among them seven metals (K, Mg, Mo [or W], Mn, Fe, Cu and Zn) are essential for sustaining life of almost all living beings, higher plants in addition need B, Cl and Ca to yield a total of (at least) 16. Some of them or their endosymbionts, e.g., N_2-assimilating rhizobacteria in root nodules of leguminoses (Co) or the fungus components of lichens (V), also require Na, Si, Co or V.

In those early days (nineteenth and early twentieth century) there were just empirical studies on growth impediments brought about by lack of some purportedly essential element, without control and counterchecks. For this purpose, Sachs (around 1882) introduced hydroponic culture method, because the composition of aqueous or other solutions can be better and more easily controlled than that of the multiphase solid "soil". Around 1900, biochemistry was extended beyond analyses of main components of biological materials into traces (e.g., determination of Ce in [animal] biomasses). Then essentiality of Fe, Mg, etc. became linked to compositions of some chemical components of (e.g.) plants, for example by identification of chlorophyll as a (porphyrin) complex of Mg.

Later on, about 1935, the first metalloproteins were isolated and identified as such (Höhne 1980). Like it had been done in earlier (though often somewhat speculative) work on pathways of sugar synthesis or nitrogen assimilation in plants, it now became obvious to compare functions of these very metal (ions or complexes thereof) in (methods and principles of) technical chemical catalysis to those observed or presumed in biological systems, e.g., Cu or V in oxidation catalysts or several metals in hydrolases. Later advances in both spectroscopy and trace analysis were to reveal the presence (and insubstitutable function) of metal ions also in many of such enzymes which had been isolated and even crystallized (rendering them accessible to both crystallographic investigation and XRF analysis) long before: one peculiar example is the identification (Dixon et al. 1975) of nickel (12 atoms per enzyme molecule!) in jackbean urease which had been crystallized by Sumner already in 1926. As for the required amounts of essential elements, there are tremendous differences both in concentrations within one species (Mg or Ca vs. Mo or Co) and among different species. This poses some problem of interpretation of the BSE: it is conceivable that corresponding differences are due to unlike "weights" of single catalytic or other functions of some metal ion among the species and taxa (giving rise to poor correlations between abundances of two elements in the set of 13 species in each case), yet some part of the observed differences may be rather due to blunt biological coordination chemistry (bioinorganic chemistry) or the necessity of organisms to reproduce in order to maintain life or the corresponding species. Both rules of bioinorganic chemistry and the criterion of reproduction (as an act of autocatalysis, chemically speaking) provide specific limiting conditions, contribute to fractionation and (possibility of) catalytic functions, or either exclude the latter.

1.1.2.1 How Do Chemical Elements Shape Biology, Biochemistry?

About one third of all the biochemical transformations in any organism, including those of nucleic acids, aromatic

compounds and nitrogen speciation forms, are brought about by metalloenzymes and thus are metal-complex-catalyzed in a certain way. Hence, the ability of anabolic metabolism is controlled by metal availability, as is transfer of elements, proper nutrition, etc. within trophic chains. Thus element flows – both bound to food and obtained from the "free" environment – can also shape ecosystems, often in a subtle way: the balance between ruminants, sheep and other hoofed animals such as deer, antelopes in open grassland or savanna (wildebeests!) may as well be controlled by the Mo/Cu ratios in soil (antagonistic toxicity to which hoofed animals are sensitive to very different extent) as by direct depletion of certain elements in grasses, leaves and other food. Among consumer organisms with different trace element demands – different in terms of both identity (stony corals need Sr while other planktivores do not, some fungi or animals do depend on administration of V or Co, respectively, while others, even closely related creatures, do not) and of amount – the abilities to settle in a certain area or ecosystems by exploiting some producer- or lower-level-consumer species living there already obviously are unlike, with chances to compensate for lacking materials from ambient water or by soil ingestion limited by either dilution of the elements or inability to mobilize them, that is, in any case, by limited or mediocre complex formation in (attempted) sequestration of the said elements (considering metal ions mainly for the moment). The elements may be constant in amount/concentration but will be differently retained or extracted, owing to, e.g., the competitive exclusion principle even though sequestration agents may be identical in rather different organisms, e.g., hydroxamates in fungi and soil bacteria.

1.1.2.2 Metal Ions and Their Relationship Towards Biocatalysis

Reactivities of other ligands such as N_2, NO or CN^- which are or get bound to metal complexes or metalloproteins are likewise influenced by the $E_L(L)$ of the coligands (Chatt et al. 1980a; Rehder 1991). Accordingly, there is also a relationship between binding properties of a central ion as defined by Eq. 2.4 and its catalytic properties which extends to metalloproteins. Thus *essentiality patterns* – including biocatalytic activities – *can be directly linked with chemical properties of biorelevant metal ions*. Another issue that arises here is whether or inhowfar features of biological uses of metal ions (biocatalysis) match the "optima" for promoting the same reaction which are known from technical or bench-scale catalytic chemistry or else differ somehow. Of course, a meaningful comparison does imply the non-biological reactions to occur in similar to physiological conditions, also. We already mentioned one conspicuous example before: transport (including reversible attachment to metal ions) of molecular oxygen in biology is effected by either Fe (haemoglobin, haemerythrin) or Cu (haemocyanin) rather than Co; many more such "discrepancies" are listed in tab. 1.1. Activation of CO_2 by coordination towards electron-rich metal centers (Ni(I), Co(I)) and/or reduction to carbonyl- besides carbonatoligands is known for long (Floriani 1983), likewise reductive terminal addition to alkene or alkyne ligands causing chain extension and eventually direct carboxylation of phenolates or carbanionoids (Li or Mg organyls). On the other hand, there is not yet a model (whether using Mg or any other metal ion) complex which mimics the function of rubisco, that is, can add CO_2 to partially oxidized organic molecules splitting their C–C backbones. The coordination chemistry of formaldehyde at V(II) [vanadocene] or Zr sites (Floriani 1983) interaction can be considered to mimic the interactions between (aldo-)sugars and metal centers even though neither V nor Zr are used for related purposes in biochemistry.

In Table 1.1, reactions or transport modes of some 30, usually small, biorelevant molecules are listed together with the metal ions which effect these reactions in (a) biochemistry and (b) catalytic inorganic chemistry, giving an impression of how abundant differences are between "procedures" in biochemistry and chemical technology even after several biollion. years of evolution. Among the substrates in this list small molecules and "simple" functional groups do prevail over larger ones or even macromolecules for the simple reason that, because the behaviour of the former is better understood also in terms of quantum chemistry, "optimum" catalysts (last column in Table 1.1) can be pinpointed there more easily. This table forms some semi-theorical background for a theoretical analysis of limiting conditions set by both evolution and geochemistry.

1.1.3 Soil and Geochemistry: Support and Storage/Buffer System for Biology

1.1.3.1 General Geochemical Considerations

Soil is also mentioned in the title of this book because it is not just a mechanical support for terrestrial plants but both a source – tapped via the roots, with or without

Table 1.1 Selected biochemical key transformations promoted (catalyzed) by metal ions and corresponding technical–chemical processes (Ochiai 1968; Riedel 2004) in comparison

Substrate	Reaction	Enzyme or carrier	Product	Group(s) of organisms which accomplish this biotransformation	Metal ions employed in biochemistry	Metal ions "best" employed in technical catalysis
H_2	Disproportionation, oxidation	Hydrogenase	H_2O	Archaea, clostridia	**Ni**, Fe (either **Ni** + Fe or Fe only)	**Ni** (PGMs)
CO	Oxidation	CO dehydrogenase (rather to be called: CO oxidase)	CO_2	Archaea	**Ni, Fe**	**Ni, Fe**, Cu
CO_2	Reduction	Formate dehydrogenase	Various		**Zn** (in clostridia: Fe + W, Se)	**Zn**
	Hydratation	Carboanhydrase	HCO_3^-	Almost all organisms	**Zn,** Cd, Co	**Zn**
CO_2	Fixation to ribulose-1,5-bisphosphate (C-site carboxylation)	Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco)	3-Phosphoglycerate (2 times)	Green plants, chemolithoautotrophs	Mg	**Ni (see above)**
	Fixation to phosphoenolpyruvate	PEP carboxylase	Oxaloacetate	Green plants (C_4 plants)	Mn	**?**
O_2/O_2^-	Transport	Haemoglobin, myoglobin, haemerythrin, haemocyanin	Transport only	Aerobic heterotrophs	Fe (-porphyrins; in animals, fungi), Cu (molluscs, arthropods)	Co
O_2^-	Disproportionation	Superoxide dismutase	O_2 and H_2O_2	Aerobics	Mn + Cu	V
H_2O_2	Disproportionation	Catalase	O_2 and H_2O	Aerobics	V, Mn, **Fe, Se**	**Fe** (solid-state reaction at oxide interfaces)
N_2	Reduction	Nitrogenase	NH_3	Bacteria (symbiotic), cyanobacteria	**Mo + Fe,** V + Fe, Fe only[a]	**W, Mo**
NO	Intercellular communication		(no product)	Metazoans	Fe	Cu
NO	Fixation to CH-acids or corresponding enolates		Hydroxamates	Fungi, (soil) bacteria	Cu	Fe, Mo
NO_3^-	Reduction	Nitrate reductase	NO_2^-	Plants, animals, soil bacteria, yeasts (if undergoing "nitrate respiration"	**Mo**	**Mo**
NO_2^-	Reduction	Nitrite reductase	N_2, N_2O or NO	Plants, bacteria	Fe, **Cu**	Mo or **Cu**

(continued)

Table 1.1 (continued)

Substrate	Reaction	Enzyme or carrier	Product	Group(s) of organisms which accomplish this biotransformation	Metal ions employed in biochemistry	Metal ions "best" employed in technical catalysis
N_2O	Reduction		N_2	Some soil bacteria	Fe, **Cu**	**Cu** (generally speaking, N_2O bound to metal ions loses O very readily)
SO_4^{2-}	Reduction	Sulfate reductase	H_2S (after several intermediates, including phosphorylation)	Sulfate reducing bacteria, plants	**Fe** (FeS cluster)	("optimum" cannot be determined as there is no function-reproducing model complex yet reported)
SO_3^{2-}	Oxidation	Sulfite oxidase	SO_4^{2-}	Aerobic organisms	**Fe or Mo** (sulfite oxidases which rely upon molybdopterin	V, **Fe**
AsO_4^{3-}	Reduction	Arsenate reductase (most often also acting as a phosphatase)	As(III), organo-As compounds	Both eucaryotes and prokaryotes, e.g., *Staphylococcus aureus*	Mo + Fe + **Zn** (combined, employing glutathione)	**Zn (?)**
Hal^- (Hal = Cl, Br or I)	Oxidation	Haloperoxidase	$OHal^-$	Several fungi, marine algae	**V,** Fe, (Se)	**V, Mo**
HPO_4^{2-}	Phosphorylation	Phosphate ligase, – kinase	(Oligo)phosphoric acid esters, e.g., phosphorylated alcohols, sugars	All organisms	Mg or **Zn** (alkalinic), sometimes Fe(II) or Mn(II) (acidic)	**Zn**
Polyphosphates, nucleoside triphosphates (NTPs)	Hydrolysis, transfer of chemical energy, activation towards nucleophiles	ATPase and similar NTPases; nucleases	Nucleoside monophosphates	All organisms	Mg	Zn, Co
Guanidine (arginine sidechain)	Hydrolysis	Arginase	Urea and derivatives, e.g., ornithine	Green plants, animals (urea cycle)	Mn (animals), Ni (green plants)	Co
Methyl groups (nonacidic)	Oxidation		$HCOO^-$ and formylated compounds		**Co**; Zn, Se	**Co**, Cu
Methyl groups CH_3X (CH-acidic, e.g., acetyl-CoA) + CO_2	Carboxylation	Malonyl-CoA-synthase and related enzymes	$CH_2X\text{-}COO^-$	All organisms (catabolic direction of Krebs cycle)	Mn	Cu
Esters, enol ethers	Nucleophilic cleavage by NH_3OH^+	Anthranilate synthetase, using chorismate, glutamine and NH_3OH^+	Hydroxamates	Fungi, soil bacteria	Mg (optional)	Fe
Methyl groups + CO	Reduction	Insertion	CH_3COO^- and esters thereof	*Moorella thermoacetica*	Ni or Cu	Rh, Co (Monsanto or Reppe processes)

aldehydes	Hydrogen transfer (either direction)	Aldehyde oxidoreductase	Primary alcohols, $RCOO^-$, esters	Yeast, animals	Yeast: Zn, animals: Mo (aldehyde dehydrogenase)	Al, Zr, B (Meerwein-Ponndorf or Tishshenko reactions)
Amino acids	Oxidative NH_2 transfer	Transaminase + pyridoxal phosphate (cofactor)	2-ketoacids, ketones; acceptors: glyoxylate in plants, ketoglutarate in animals	All organisms	No metal	(various degrees of **inhibition** by metal ions [slight with Al, Cu, Zn, pronounced with Fe, REEs])
	Polycondensation	Peptide ligase	Peptides, proteins	All organisms	Zn	Cu
N (nonbranched) carboxylates (fatty acids)	β-Oxidation (abstraction of acetate units)	Shorter-chain carboxylates, eventually: acetate or formate	Acetyl-coenzyme A	Most organisms	**Fe** (bound in ironthio-clusters (ferredoxines)	Thioclusters $\mathbf{MFe_xS_{3,4}}$; identity of M does not matter much
Sugars	Glycolysis: involves phosphorylation and C–C cleavage of sugar molecules	Sugar kinases, etc.	Terminal product: pyruvate	All organisms	Mg	Cp. Phosphorylation (Zn)
Pyruvate	Decarboxylation	Pyruvate decarboxylase	Ethanal	Yeast, some vertebrates (e.g., *Carassius auratus*)	Zn	Cu
	Oxidative decarboxylation		Acetyl-coenzyme A	All organisms		
electrons	Transportation towards some reducible sink	Quinone oxidases and others	Reduced electron acceptor (e.g., water)	All organisms	**Fe** (quinone oxidases); **Fe + Cu** (cytochrome c oxidase)	**Fe + Cu**
Citrate, etc.	Redox cycle which links oligocarboxylates and involves absorption or formation of CO_2	Enzymes of tricarboxylate cycle		All organisms	Fe (aconitase), Mg (succinyl-coenzyme A synthetase), **Mn** (malate oxidoreductase)	For aconitase behaving as anisomerase: Co; **malate oxidoreductase: Mn**[b]
Benzenoid or polycyclic aromatics	Hydroxylation	Various	Phenols, hydroxi-PAHs	Most organisms	**Cu** (laccase), Fe (haem peroxidases)	**Cu**

[a]When reacting with nitrogenase, N_2 and its other substrates (nitriles, isocyanides, ethyne, etc.) get bound to Fe while "model compounds" reducing N_2 at ambient conditions may also contain Fe but rather accomplish N_2 trapping and reduction at Mo, W, V or Re sites (Schrauzer 1975; Chatt et al. 1980b; Rehder 1991).

[b]Although Cu would be a more efficient oxidation catalyst, it does also support decarboxylation much more than the lighter 3d-ions. As a result, malate would be converted into pyruvate rather than oxaloacetate, causing the entire tricarboxylate cycle to collapse. As Mn^{2+} does hardly cause decarboxylation (Pedersen 1948; Hedrick and Sallach 1961), but favors oxidation, it affords a kind of local optimum for this purpose.

Bold letters: Metalloenzymes and technical catalysts agree with respect to given reaction.

assistance by other soil-dwellers like mykorrhiza – and a sink to chemical elements which make their way through some plant before recycling by foliage littering or crackdown of the stem of some tree. This corresponds to a set of element cycles which are usually but partly closed, efficiencies and chemical details being subject to all plant cover, mineral composition of soil and succession/soil stratification during development. In fact, soil is the principal source of elements other than C (atmosphere), sometimes N and S (air also, plus rainwater in polluted areas) and some metals from dust. As we do here compare concentrations of elements in soil and plant (parts) to determine bioconcentration behaviour (BCF values), some general remarks on soil, its origins and vertical structure are due here. By mass, most of soil is composed of mineral phases, often with substantial shares of silica (sand, etc.) and clays, besides metal oxides like Fe_2O_3, Fe_3O_4, MnO_{2-x}, carbonates, phosphates, silicates, etc., with minor shares of water dissolving some salts, organic components and eventually gases (air or reducing gas phases). The organic components, much of them humic acids with phenol, carboxylate, 3-ketoenolate and other functional groups, can bind metal ions, partly leaching them from the mineral phases, partly withholding them from plant roots which produce metal ion sequestrants of their own. Their share, approximated by weight loss during aerobic heating to 500°C, usually is <10 % by mass.

Thus, soil is a chemically – and biologically – highly active multiphase system – which always must be considered as a chemical reactor linked to both plants and hydrology (e.g., springs, creeks, etc. in forests) in order to understand chemical dynamics in individual green plants and larger ecosystems alike and to model it. Moreover, it is dynamic also with time, changing its chemical features often within rather short periods of time, e.g., when a bog converts to solid land, with the assembly of plants undergoing thorough concomitant changes.

In the broad sets of data for soils in different climates and geochemical impacts, e.g., by tillage, the focus mostly rests with elements which are essential to plants which limits the chances to obtain data on fractionation or information to which extent retention of elements is simply due to complex formation in soil. As an exception, element distributions down to –4.0 m from litter level were investigated at Bornhöved (Schleswig-Holstein, northernmost part of FRG) test site (Arenic Umbrisol) also for Al and Ti which both merit particular interest for their combination of high abundance and non-essentiality. In this rather acidic soil a distinct minimum of both Al and Ti (which is considered immobile there) exists in the B horizons under forest between 60 cm and 1.7 m depth (Fränzle and Schimming 2008) while concentrations of Mg, Ca or Mn agree with those above and below except for single, sharply confined enrichments. Accordingly, when sampling plants the roots of which descend to ≥1 m beneath the soil surface external ratios of both elements which readily form carboxylate complexes (Al/Mg, Ca) and others which do not (Ti, Mn) will differ from those elsewhere. Obviously Al and Ti are taken up or otherwise removed from down there. The change in ratios corresponds to a change in plant ratios and in turn to attributions to element clusters of identical BCF used to calculate $E_L(L)_{eff}$. While Ti rarely is contained in any such cluster, although being absorbed by plants, the problem is more pronounced with Al. Regrettably there are no values for REEs from Bornhöved (either leaves or soils), keeping in mind that most of them correlate very strongly with Al abundances in plants.

Soil does vary in chemical properties among the different levels while, as a rule, plant roots on solid land penetrate just through oxic layers, except for wet stands (*Alnus*, etc.). Likewise fungi which degrade rotting wood or even lignite can do so only with dioxygen being available. Hence element takeup and partitioning between supporting solids – as a rule, soil – and plant or fungal biomasses will refer to those oxidation states and speciation forms which are stable in presence of O_2, e.g., MoO_4^{2-} rather than thiomolybdates or Mo(III). Nevertheless, reduced forms may be produced and deposited within biomass even if the latter is coupled to air or (by photosynthesis) even produces O_2 itself (e.g., magnetite forming in leaves of green plants [Fränzle et al. 2009]) or become part of bioactive speciation forms (thiomolybdates linked to pterine; Fe(II), Ni(I) in enzymes). This process removes the corresponding metals from the previous state of equilibrium. The bioconcentration factors then are influenced by such secondary reactions, as they are by membrane permeation and the kinds of ligands roots or mycelia give away to soil, to recover the metals along some part of these materials by back-resorption. Metal-processing bacteria are far more effective in metal

turnover in soils, retrieving more than 95% of the compounds delivered to soil, thus cleaving most of Fe(III) then being used as a biological oxidant. In this case, the relative N content of the sequestering agents (siderophores) considerably surpasses that of the remaining biomass (which is hardly relevant given the extent of re-absorption), in stark contrast to the situation with higher plants (≠ grasses) and fungi. In ferric reducers (microorganisms), stoichiometric ratios C/Fe are of order one (Fränzle and Noack 2008), rather than $>10^4$ in "colloquial" plants, and sequestering agents which dissolve and give access to Fe(III) [polyphenols, hydroxamates, generally siderophores] contain about 20 carbon atoms. To maintain the above C/Fe ratio, >95% of the produced siderophore Fe complexes must be really retrieved by the organism.

Among the solid metal oxides (sometimes) accumulating in B horizon as mentioned before, MnO_{2-x} is of paramount importance as a redox catalyst which both will change oxidation states, mobility, bioavailability and toxicity of many metals (Cr, Hg, Ce, V) and non-metals likewise and alter metal binding properties of soil organics by, e.g., cleaving polyphenol sites in soil aromatics (humic acids). Of course, Ru complexes of humic acids (of low molecular weight, like amino acids and other carboxylates, phenols, hydroxamates, etc.) were also investigated with respect to their redox potentials with the end to estimate complex formation constants.

Generally speaking, matter transport in soil is slow (ground water tends to move a few m/year), permitting construction of steep chemical gradients inside soil which influence all mobility, speciation and bioavailability of quite a number of chemical elements. Elements may be kept from takeup from certain soil horizons, or stick to them in vertical transport or be volatalized subsequent to hydride or alkyl formation (Wood 1975; Thayer 1995).

Under strongly reducing conditions, several transition metals may also be mobilized – from decomposing biomass or beginning with certain enzymes such as hydrogenases as precursors – as homoleptic, volatile carbonyl complexes (Mo, Ni, W, not Fe); this reaction (Feldmann 1999) thus extends beyond the small range of transition metals which directly form carbonyl complexes upon contact of the bulk or dispersed (dust, amalgam) metal with CO (Elschenbroich and Salzer 1988).

1.2 Methodology of Inquiries into the Biological System of Elements

1.2.1 *Correlation Analysis of Element Distribution in Multiple Plant Species*

Data reported by, e.g., Bowen (1979), Markert (1996) or Emsley (2001) give an idea on the common ranges of variation of concentrations of most stable (non-radioactive) elements in higher plants, concentrations of some elements are fairly constant while others vary over several orders of magnitude, without any relationship to possible functions like biocatalysis.

In addition, elementary analytics as broad-scoped as this allows for comparisons of metal concentrations beyond taxonomic borders as well as those of spatial, bioclimatic distributions. These comparisons eventually (1989) were merged into a complete set of abundance correlations among 45 chemical elements (including non-metals like B, Si, Cl or Br) for 13 plant species or parts thereof. Markert called this set of abundance correlations the "Biological System of Elements" (BSE, Fig. 1.3).

Owing to the (usually) lower pH of freshwater, avoiding hydroxide or aquoxide precipitation, there is an increase of concentrations of metals forming insoluble (hydr-)oxides with fresh water, which also bears ecochemical implications. This holds for, e.g., most transition metals. From this starting point pathways of chemical and early biological evolution (concerning uptake and usage of metals at least) can be reconstructed.

Returning to the empirical BSE patterns and the list of essential (biocatalytic metal) elements, metal ion properties sensitivity and intrinsic binding stability allow for a relationship to quantitative parameters. How, then, do essential metal ions differ with respect to complex formation stabilities with biomass and/or biological substrates and/or c and x values from those which are not essential (e.g., Al) or others which, although essential, do not directly promote reactions (e.g., Ca)? If parameter sets obtained from Eq. 2.4 can be linked to biological features such as essentiality or abundances in biological materials, the abundance correlations comprising the Biological System of Elements (Markert 1994a, 1996) could be traced back to chemical

properties of plant biomass (general or species-specific ones) *without* needing to know details on chemical methods of transport or binding of these metals. The lack of structural information would not matter so much because the number of different metal-ion-binding groups in biological matter is rather small. This is corroborated by the overview of methods used in this investigation, which are listed and discussed in the forthcoming Chapter 2.

Analogies between the Biological (BSE) and the "classical" chemical (i.e., periodic) system of elements (PSE) are partly due to the fact that similar chemical properties can (not must) bring about common enrichment in plant biomasses. Yet, the biological system of elements does not give another representation of chemical similarities identical to that contained in the PSE because biological functions (or toxicity, respectively) cause selective or even specific reactions towards supplies by certain elements, either binding them more strongly or inactivating or keeping them from the organisms altogether (e.g., by means of phytochelatins or chaperons). Notably, even though the elements get directly linked, with some of these links denoting very strong correlations in the triangular picture above, sometimes also corresponding to chemical similarities, Fig. 1.3 yet does *not* reveal something like "biological groups of elements". On one hand, there are highly correlated abundance distributions among chemically similar elements like REEs (for definition see below), but this also holds for much less similar pairs of elements (e.g., yttrium and vanadium), whereas conversely members of the same group of chemical elements sometimes may not display any statistically meaningful abundance relation to each other; cases of this are the couples P/As (r = – 0.146) or Ca/Ba (r = 0.231).

Owing to biochemical features and processes, there is additional information which distinguishes the BSE from both the PSE and from geochemical descriptions of the elements (Railsback 2003). By the above "deviations" from expectations suggested by the PSE or similar ionic radii, the Irving-Williams series, etc., the BSE in addition contains information on processes of transport and inter-metal fractionation in plants (or other organisms). For example, Irving and Williams (1953) or Sigel and McCormick (1970) point out that complex stability constants and coordination polyhedron geometries of Co^{2+} and Zn^{2+} are most similar to each other, sometimes even allowing for effective replacement of one ion (mainly Zn) by the other in catalytic properties of metalloenzymes (Vallee and Williams 1968). Yet, their abundances in the above set of 13 plant species are entirely unrelated (r = – 0.092). Thus a chemical "fingerprint" is obtained which allows for functional statements based on useful parameters of bioinorganic chemistry, which will be defined in this book lateron.

1.2.2 Fundamentals of the Correlation-Chemical Analysis of Element Abundances

It is not sufficient to investigate amounts and distributions of chemical elements when considering biological materials; an understanding in terms of chemical biology rather needs additional questions to be adressed, including those on functions of trace components (related to essentiality), whether their administration is indeed required to maintain life and fertility, eventually which amounts are required for these purposes.

Corresponding binding and transport processes in turn can be inferred from studies of and similarities with respect to abundance distributions (including BCF values and clusters thereof) both inside some organism and when comparing different species. There is one large group of ubiquitous chemical elements which perfectly match these conditions although, due to common BCF values soil/leaf of order 10^{-3}–10^{-2}, only the more abundant ones (Y, La, Ce, Pr, Nd) can be readily quantified in terrestrial (Markert 1996) or aquatic (Cowgill 1973; Weltje 2003) plants: the REEs (lanthanoides). For this discussion, REEs = La – Nd, (Pm being irrelevant for being a short-lived radioelement [$T_{1/2} \leq 18$ years]), Sm – Yb (Z = 57 – 70), including Sc and Y. As *lutetium* (Z = 71), commonly counted among the REEs, does neither make use of 4f orbital states in redox reactions [there are no Lu(IV) compounds in condensed matter] nor does so in coordination chemistry (it prefers hexacoordinate states to the higher CN values [8 – 11] common in "real" REEs, owing to an irreversibly filled $4f^{14}$ state) and correspondingly gets fractionated from other REEs concerning its correlations to abundances of e.g., Al or V (there is no abundance correlation Lu/V whatsoever, while REE/V or Y/V are highly correlated) and Lu is

enriched together with Ca – unlike La...Yb –, accordingly Lu is not counted among the REEs here.

Otherwise abundances of all essential, non-essential and toxic elements in different plant species were measured and compared (Bowen 1979; Kabata-Pendias and Pendias 1984; Markert 1996) with the latter author calling the inter-species abundance correlations derived from these analyses the Biological System of Elements (Markert 1994a). Besides the REEs and Y, abundances of yet other metals (Al, Ti, V and essential Fe) are linked to each by very highly positive correlation coefficients (Markert 1996).

The present work and book deal with identifying factors which contribute to essentiality in the above manners, trying to put these into quantitative terms if possible. There are three different sources of theoretical reasoning:

- *Stoichiometric network analysis* (SNA)
- *Quantitative* arguments from *coordination chemistry*
- Biochemical applications and implications of *Gibbs's phase rule*

which combine to yield the "*rule of three functions*".

These theoretical frameworks were selected for formal integration and "explanation", making no assumptions other than the ability of living beings to reproduce and that some chemical features of (biocatalytically) essential can be pinpointed which distinguish them from non-essential elements. "Explanation" here means reduction to some theoretical framework in quantitative terms also, that is, constructing a model which can account for the observed effects vs. exclusion of others which are not observed, e.g., non-essentiality of some other elements owing to a semi-empirical description of their chemical properties.

1.2.3 Stoichiometric Network Analysis

Going beyond comparisons of element abundances in various green plants (and possibly, or to some part, also in other organisms, trying to understand food web-based transport of elements possibly and actually controlling essentiality patterns in either participant of a trophic relationship), which may or may not be related to similarities in biochemical pathways, there are also more fundamental (and thus general) principles from chemical physics which likewise apply to living beings and the ways they (can or cannot make) use certain chemical species. The principal feature of living beings controlling their use of matter including chemical elements in metabolism – besides spatial heterogenity – is autocatalytic feedback produced by reproduction or simple cell-budding.

The principal challenge in *"explaining" the BSE* is to account for the relationship between enrichment (bioaccumulation, biomagnification) and function (if there is any). Table 1.1 (below) shows the remarkable abundance of "discrepancies" between those metal ions identified by empirical technical catalytic chemistry to promote/accomplish some transformation most effectively and those used as biocatalysts for these very reactions in biological systems even after almost four billion years of evolution; evolution processes as a rule identify local rather than global optima in the event space even though they can discriminate rapidly among $>>10^{50}$ possible configurations (Rechenberg 1973). Yet this apparently does not imply that the optimum among 30 or 35 possibly suitable and fairly abundant metal ions can be localized in the spatiotemporal frames of terrestrial biological evolution.

1.2.3.1 Biophysical Implications of Gibbs's Phase Rule

Biological systems, in general, are heterogeneous systems, heterogeneous both with respect to coexistence of liquid and solid phases and to chemical compositions of either; however, one cannot increase corresponding complexity arbitrarily without analogous increases in chemical complexity, otherwise the system (that is, the organism) would not be sustained. Because amounts or shares (e.g., for Mg in vertebrates) of metal ions used for catalytic purposes tend to be very small, an enrichment in biological materials can only be identified by correlating it to ligand properties capable of modelling biomass. Empirically, some correlation of abundances and bioconcentration/biofractionation with the electrochemical ligand parameter (of the protein molecule for this case) allows to define *effective* parameter values. These effective values apparently can be treated and used as if a single kind of substance or donor group be responsible for interaction and fractionation in an organ of an organism, and likewise for intracellular enrichment, toxic effects or such obstructing reproduction (Fränzle et al. 2005).

Generally speaking, this is to say that fractionation among metals obtained from soil or water (in aquatic organisms) behaves like that caused by a single kind of immobile ligand even though there are several changes of speciation during uptake and intraorganismal transport. Though the values thus calculated do not depend on environmental burdens in *Betula alba* at least (Fränzle and Markert 2006a), there are differences due to (a) "abnormal" speciation of some element included in a BCF cluster [often Cr which probably was oxidized to chromate (VI) in the soil and thus absorbed in a different way and extent, placing it into another cluster of BCF values] and (b) with age of photosynthetic organs (e.g., in first- and second-year needles of spruce *Picea abies*). The ecochemical implications of such differences – beyond those which mark "deviating" oxidation states and thus soil chemistry, e.g., catalyzed by MnO_2 – are still to be investigated. For $E_L(L)_{eff}$ of different parts of the same plant (*Pinus sylvestris*), see below. Table 1.3 includes the other kind of value required to do this kind of analysis, namely the electrochemical ligand parameters of biological substrates and other biorelevant ligands.

From this combined approach one obtains both a contribution towards an understanding of reasons of essentiality and an idea why there are elements which are both very abundant and suitable for some catalytic transformations (Al, Ti; consider, e.g., hydride transfer, the multitude of transformations catalyzed by clays, backbone of minerals) yet do not exert any biological function in any organism. Eventually, the relationship between quantitative aspects of coordination chemistry – given in a novel scale – and essentiality of elements, given in mappings, will also be discussed in the framework of chemical and biological evolution.

1.2.3.2 Aqueous Coordination Chemistry Related to Metal Uptake

This method to determine metal fractionation by attributing/identifying binding properties/selectivities to or of biomasses due to their similar ways of interaction with metal ions of course includes some assumptions. One assumption relevant for metal ions which can exist in various speciation forms in the environment concerns the formal oxidation state because the c and x values depend on the latter. Thus, attributing some "wrong" oxidation state to a member ion of a BCF cluster will produce a value of $E_L(L)_{eff}$ which differs from those obtained with other clusters of metal ions producing identical BCF in the same taxonomical species. One element for which this frequently occurs is chromium. Whereas Cr(VI) – CrO_4^{2-} – will be resorbed by the sulfate (anion) transporter and thus is likely to resemble sulfate (or molybdate(VI)) with respect to BCF, Cr(III) does form less stable complexes than (e.g.) Al(III) and thus should be less enriched provided $k' > 0$ (cp. Section 2.2.4, for definition of k' as an internal, membrane-transport-related amplification factor beyond "mere" biocoordination chemistry see Eq. 2.11 combined with the central Eq. 2.4). Similar "deviations" from colloquial kinds of metal uptake and –transport are seen with vanadium and should also occur with U, As or Ce. Thus one can detect whether Cr exists as either CrO_4^{2-} or Cr(III) around the rhizosphere of the plant used for biomonitoring, which is of obvious ecotoxicological importance given the differences in both resorption and toxicity of Cr(VI) vs. Cr(III) for humans and a manifold of animals alike (including dermal resorption of CrO_4^{2-}; the primary step of uptake occurs without a redox reaction although some >70% of CrO_4^{2-} undergo reduction already within the root region; so, the argument keeps valid).

Since the equilibrium between CrO_4^{2-} and Cr(III) is established by MnO_2 catalysis, the local geobiochemical situation in an aerated soil may be inferred from those "deviations" concerning clusters which contain Cr or V or Ce. Examples are given in Table 1.1 below:

There may be similar effects with reducing, fully wet soils and reduced oxidation states of V, U, Mo or other metals.

Another feature which is indicative of (the role of metals in) biochemistry in higher plants or algae or fungi is represented by the responses to very *high salinity* levels (>>100 g/l NaCl). Then, although photosynthesis – both that variety oxidizing water and that making use of sulphur species (done by cyanobacteria and thiorhodacees) – can still be accomplished in saturated NaCl or $CaCl_2$ (Siegel et al. 1984) brines,

- All ammonia, urea or nitrite cannot be oxidized to yield NO_3^- anymore.
- Acetate, lactate or longer-chain fatty acids do no longer undergo oxidation by sulfate.
- CO_2 hydrogenation to afford CH_4 is inhibited.
- decarboxylation of acetate (Oren 2001).

On the other hand, CH_4 can still be cleaved from methanol, methyl amines, glycine or sarcosine (Oren 2001). Accordingly, hydrogenases (Fe and/or Ni),

sulfate reductases (Fe thioclusters), acetate splitting enzyme (Cu?) or formate oxidase (Zn) respond to very high chloride levels by inhibition while methane production relying on W does still operate, likewise water oxidation can be achieved at Mn sites. This obviously corresponds to different (relative Cl^-/substrate) binding stabilities and thus different x_{1d}-related selectivities of the involved metals.

There is only some subset of the essential metals which can still fully operate in such conditions; in addition, higher plants (other than algae, cyanobacteria and some lichens) will not survive and do photosynthesis if exposed to solutions much beyond marine salinity and chloride levels. With chloride penetrating into the cells, there is an example of competition which limits chances for biology, supporting the idea on what kind of factors does control essentiality: besides very high temperatures and low redox potentials, there is a third realm of favorable operation for tungstoenzymes. Chloromanganates likewise are so labile that water photooxidation will not be jeopardized.

Chapter 2
Autocatalytic Processes and the Role of Essential Elements in Plant Growth

There is no direct relationship between essentiality patterns of chemical elements and analytical data for biological materials: while any organism selects some (dozens of) chemical elements for running its biochemical functions, several of these may be present, both required and tolerable at very low concentration levels only (V, Se) though essential whereas there are substantial amounts of non-essential metal ions in the same organism (Al, Rb, Sr, Ti, etc.). There are also differences with respect to biochemical effects. In additions, elements do interact during uptake: regardless whether an element is essential by itself, it may influence the uptake of another – regardless whether essential or non-essential – one, e.g. by competing for the same carriers. There also are changes of effects (Fig. 1.2) which can be detected by changes of plant growth rates.

2.1 Biomass Stability in the Light of Gibbs's Phase Rule

All organisms both consist of a large number of chemical components – which cannot be freely interconverted as they contain many other different elements besides C, N, O and H, including many metals which are in the focus of interest here – and have some internal structure, with membranes inside and possibly in between cells controlling physical and chemogenic diffusion and charge transfer likewise. Coupling between either phenomenon is essential for retrieval and storage of chemical energy in biochemistry. Hence, there are multiple discernable phases inside a single organism which implies – since there is exchange and chemical coupling – their long-term coexistence be influenced by phase rules from physical chemistry. The principal criterion is the number of degrees of freedom – capability to adapt to varying external physicochemical conditions – which determines either "specialist" or euryoicous properties of the corresponding (taxonomic) species. This is thus directly related to chemical complexity which yet cannot be increased arbitrarily for other limiting conditions from physical chemistry (three-functions-role, see below).

Now, in some metazoans dynamic biochemical stability or number of degrees of freedom might be increased by reducing the number of different organs and tissues but, as a rule, this cannot be done without grave consequences for biological function. Rather, the number of components might be increased in secondary metabolism (at least that of heterotrophs) to meet the phase rule demands, that is, some part of metabolic "independence" which was gained by autotrophy may be "traded" for better stability, at least temporarily. The situation is most grave during reproduction while in metazoan plants during reproduction the early states – seeds, seedlings, etc. – do not yet act as autotrophs (with light being excluded by soil or litter covers, additionally) but depend on heterotrophic degradation or transformation of some stored materials (starch, lipids and so on). Certain organs will form only afterwards, so complexity is both "delayed" or "saved" and reduced with respect to its implications.

Among the compounds that add to the list of essentials there are secondary metabolites including essential organics, e.g. antibiotics in fungi can be produced from phenylalanine (Habermehl et al., 2003). Alkaloids or antibiotics thus obtained do not just represent toxins protecting against being eaten but also contribute to the dynamic stability of this autocatalytic network. Another interesting feature would be enrichment of such chemical elements from the environment which otherwise lack biocatalytic, structure-supporting, information-carrying or other obvious functions, like Rb, Sr or Al which all can attend substantial concentrations in

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plants (Farago 1986; Marschner 1986; Markert 1996; Kabata-Pendias and Pendias 1984). By their mere presence they tend to stabilize any reproducing system, providing additional degrees of freedom, accumulation granting their effective permanent presence throughout the generations. If, however, K can be fully replaced by Rb like in many algae and bacteria (or, somewhat more hypothetically, Ca by Sr) the number of "really" essential elements will decrease accordingly, and so will the number of stably co-existent phases (organs, tissues). **Hormesis** – which is a fairly common effect in plants and animals – or the production of additional species under stress condition can be rationalized by the same argument: the number of components *K* is increased by supply (and be it intoxication by some detrimentally biochemically active substance!) or endogenous production of additional stuffs even though these might become eventually toxic, thus there is no need to reduce *P*, in favor of the integrity of the organism. Hormesis thus occurs, stimulating metabolic activity in some organism, increasing its growth rate, etc. when small amounts of some otherwise toxic substance are administered.

Table 2.1 gives an overview that shows there is no dramatic increase of the numbers of essential elements with histological complexity even though in metazoans it is somewhat larger than with bacteria, archaea or protists. However, more highly organized heterotrophs may gain additional degrees of freedom which they require by introducing more essential elements; conceivably this did happen not only in endosymbiotic organization of first eucaryota about two gigayears ago but also later on as animals make use of more essentials than other metazoans, supporting fulfillment of Gibbs's phase rule besides their lacking ability to synthesize many functionalized products on their own.

It can be directly derived from the **rule of three functions** (see below, Section 2.2.7) that three substrates (it might suffice that these are different enantiomers or diastereomers) correspond to three enzymes which can be distinguished in both functional and chemical terms. For each essential element, there thus must be at least four substances (three solid or dispersed/membrane-attached enzymes and the very speciation form of the essential element in dissolved or atmospheric (N, O, C, H, S) states) in the organism or directly linked to it in the environment (soil, water, food, for photoautotrophs also the atmosphere (CO_2)). Living beings share one more property with "traditional" multiphase systems: the different phases (i.e., distinct kinds of cell, see Section 3.3) must differ in terms of chemistry rather than just exist as either gaseous or condensed states. The number of these phases tends to be considerably larger in animals than in plants, often being around 100. This implies some similar chemical complexity which – at least for higher animals – can no longer be realized just by an array of chemical elements introduced into (catalytic or other)

Table 2.1 Essentiality of chemical elements in the biological kingdoms; xx means "generally essential within this group", x means "known to be essential for one or some member(-s) of the group while p denotes "purported" or "presumed" which is to say essentiality was made likely by depletion experiments followed by controlled administration of the corresponding element (data from Markert 1998; Emsley 2001)

Element	Algae	Fungi	Vascular plants	Animals	Bacteria	Archaea
H	xx	xx	xx	xx	xx	xx
Li				p		
Na	x		x	xx	x	?
K	xx	xx	xx	xx	xx	xx
Rb				p		
Mg	xx	xx	xx	xx	xx	xx
Ca	xx		xx	xx	x	
Sr	x			x		
Ba	x[a]			p		
B	xx	x	xx	p		
C	xx	xx	xx	xx	xx	xx
Si			x	x	x	
Sn				x		
N	xx	xx	xx	xx	xx	xx
P	xx	xx	xx	xx	xx	xx
As	x			x		
O	xx	xx	xx	xx	xx	xx
S	xx	xx	xx	xx	xx	xx
Se			x	xx		x
F				x		
Cl	xx		xx	xx	x	
Br	x					
I	x[b]			xx		
Cu	xx	xx	xx	xx	xx	xx
Zn	xx	xx	xx	xx	xx	xx
V	x	p	x	xx	x	
Cr				p		
Mo	xx	xx	xx	xx	xx	xx
W					x	x
Mn	xx	xx	xx	xx	xx	xx
Fe	xx	xx	xx	xx	x	xx
Co	x			xx	x	
Ni	x		x	xx	x	x[c]

[a]Desmid algae

[b]Some marine algae

[c]Methanogenic archaea (Ni as nickel porphyrin (F-430 enzyme))

chemical elements; rather it requires additional indispensible species which might arise the easier as animals are not autotrophic and thus less capable to achieve thorough chemical transformations around some elemental center: many things which are possible to plants or autotrophic bacteria cannot be done by animals or fungi, bringing about the necessity of various cofactors (e.g. folic acid for methyl group synthesis and transfer) and in turn increasing the number of chemically different "essential" entities. Of course substrates of enzymes also belong as components to this system but cannot be quantified as easily as they undergo stepwise transformations by multiple enzymes. Nevertheless principal components include those amino acids which cannot be produced by the corresponding organism (from zero in fully autotrophic organisms to a considerable part of the 22 proteinogenic ones (e.g. eight in man)), specifically acting other carboxylic acids (unsaturated lipid fats), nucleotide bases, certain sugars including cellulose or chitin, dissolved nutrient like phosphate, sulfate, oxidized or reduced N species constitute a stockpile of 50–70 components together with the essential elements. The theoretical minimum for some fully autotrophic organism (be it phototrophic or chemolithoautotrophic) would be constituted by its, say, 15 essential elements. Now Gibbs's phase rule provides the extent of "sustainable" histological complexity or – in metazoans – also biochemical differentiation from this numerical chemical complexity, plus liquid phases (oil drops and aqueous liquids inside plants – e.g. in xylem or seeds – and their roots) and possible mineral phases such as SiO_2 needles, SiO_2 or $SrSO_4$ exoskeletons in certain algae or magnetite Fe_3O_4 (Fränzle et al. 2009) within leaves. Thus histological complexity of green plants is effectively limited by autotrophy itself, which fact moreover appears to have precluded the evolution of chemolithoautotrophic metazoans altogether. For animals the range of complexity is from two to three different kinds of cells in sponges up to some 200 in man and other mammals.

Given that Gibbs's phase rule does produce some drawback between complexity and biochemistry, with the complexity seen in higher animals only feasible with substantial lacks in biosynthetic capabilities and correspondingly high dependence on external acquisition of already highly sophisticated compounds (e.g., vitamins), "construction" or evolution of complexity by histological differentiation – e.g. in formation of sensory organs – always bears some risks. Deviation from former autotrophy, like in fungi and certain parasitic plants, on the other hand, offers a chance to benefit from increased structural differentiation whereas photochemical or lithotrophic usage of organics (oxidation by Fe(III) reduces them to the role of an electron source (Hersman 2000)) which is more accessible than water while the former structural complexity does not matter anymore: when it is irrelevant whether a lithotroph oxidizes methanol, toluene, or some component with multiple stereogenic centers such as isoleucine or even a polymer for energy production without integrating stereochemical information or trace metals from that material (e.g. metals bound to chitin) the corresponding potential for producing more internal complexity while obeying Gibb's phase rule is essentially given away and lost. Hence, once again, such organisms tend to "remain" unicellular.

2.2 Coordination-Chemical Control of Element Uptake

2.2.1 Metal Complexes in Biology: Definition of Complex Formation Constants

Complexes form from parts of biomass, more precisely, metals will interact with donor sites provided by both polymeric (proteins, nucleic acids, polysaccharides, chitin, hyaluronic acid (the latter in fungi, animals), glycolipids, etc.) and monomeric (amino acids, hydroxycarboxylic acids, oligophenols (tannines), reduced sulfur compounds, hydroxamates (in fungi, bacteria), isocyanides (in sponges), etc.) species; this is to say that biomass (also) bears ligand properties. The metal ions can be admitted from outside or retained within some plant. Thus, the first objective of this work was to produce a generally applicable scale to determine and then calculate complex stabilities from properties of both metal ions and ligands to determine bioinorganic element speciation. Scales which were prepared and published earlier (e.g. Misono et al. 1967; Nieboer and McBryde 1973) often contain too many empirical parameters each of which holds for just one single kind of ligand rather than an entire class of donors, e.g. referring to glycinate only rather than the full set of aliphatic amino acids,

although complexes of all these are very similar in hydrolytic stability (Irving and Williams 1953; Kiss et al. 1991). Thus a prediction for cases in which chemical details are not known is not feasible using such increment scales, neither a description which relates metal retention and fractionation to certain ligand properties.

In higher plants, resorption of 3d-heavy metals (that is, neglecting Sc, Ti, and Mg, [Al] besides various non-essential ones like the REEs but not Mo) is effected by complexation using citrate, malate, oxalate and certain amino acids as ligands (Farago 1986; Marschner 1986; Scheffer et al. 1998). For the non-amino-carboxylates, both structures and $E_L(L)$ values are fairly similar, the latter being between −0.13 and −0.25 V (Lever 1990; Fränzle 2007 and this work). The metal ion distributions and BCF values (Tyler 2004b) observed in plants of forest biotopes – not just in trees – can be linked by regionalizing statistics to the local environmental emission situation (Pesch and Schröder 2006). It is rather difficult to clean roots from adhering soil sufficiently to obtain meaningful in-root metal concentrations without removing the outer root layers altogether; hence the focus of presently available data (also on the corresponding ecological stoichiometry, is with assimilation organs (Schröder and Fränzle 1992; Pfennigsdorff et al. 1993)). This, however, implies that the measurements correspond to data and sites which – except for fruits and seeds – are at the extreme end of the entire cascade of transport- and speciation processes afflicted on some metal ion on the way into and through a plant (Farago 1986; Clemens et al. 2002). For plants, use of the above ligands modifies relative concentrations of the essential ions in soil in a way which allows for appropriate supply with metals while fungi – for being heterotrophic – need rather different relative amounts of the same (and other – e.g. vanadium) essential elements which, given the relationship between $E_L(L)$ and metal ion fractionation expressed by Eq. 2.4, can be met by delivering ligands of higher $E_L(L)$ such as hydroxamates or amino acids or an combination thereof.

As the composition of soil is less constant than the demands of any such plant or mushroom species and there are considerable metabolic expenditures in making and exmitting the above ligands parts of which cannot be retrieved and/or remetabolized again (thus are "real" expenditures), any such strategy is just a biochemical compromise which is different in plants and in soil bacteria or in fungi which, besides hydroxamates, make use of polyphenols, diketopiperazines (cyclic dipeptides) and others. For oxidized topsoil layers, iron (thus Fe(III)), copper and vanadium are more accessible to fungi than to green plants – represented by a beech tree in Fig. 2.9 – while fungi hardly use calcium and have poorer access to Mg and Mn which are of larger importance to green plants for their principal role in photosynthesis (Kitao et al. 1997). Mn is thus used in fungi mainly in a very particular way which escapes the problem of appropriate up-take: it is used in and by exoenzymes (exoperoxidases) without absorbing it into the organism at all before. V which is not required by higher plants has its role in fungal redox proteins or peptides like the haloperoxidase from *Curvularia inaequalis* (Table 2.1), likewise Ca is not needed by most fungi (Emsley 2001). Figure 2.9 demonstrates the results of different metal ion sequestration selectivities in green plants and in fungi; the positions of the tree and the (fly agaric) toadstool refer to weighted average $E_L(L)$ of the involved ligands while invididual values of which ligands are given by their written position (vertically) in the diagram.

From the onset of resorption by roots the binding of metal ions to plant biomass is mainly controlled by complex formation equilibria. For an estimate of complex formation properties in biomass then determination of electrochemical ligand parameters controlling complex stabilities according to Eq. 2.4 is to be followed by calculation of the c and x parameters of both essential and non-essential metal ions in the oxidation states which are physiologically relevant and stable in biochemical environments. For the sake of comprehension, c and x parameters are eventually linked to essentiality and other corresponding properties of an element in mappings (Figs. 3.1 and 3.2). These diagrams reveal some gathering of essential elements around certain values of c and x. Let us now embark on quantification of the relationship between ligand properties and complex stability as determined by the central ion.

2.2.2 Electrochemical Parameters of Biologically Relevant Ligands

Stability of chemical bonds between ligands and metal ions corresponds to some state of thermodynamic equilibrium – which is to be described in quantitative

terms – among different complexes with differing chemical surroundings because the environment can also interact with metal ions: first, there is water, besides it other compounds which may react with metal ions, including metal ions in the centers of proteins, such as substrates of the said metalloproteins or functional groups in side chain of amino acids. In coordination chemistry, corresponding chemical equilibria are given as complex formation constants $k_{ass} = 1/k_{diss}$ which represent the relationship between amounts of aqueous metal-ligand complex $[ML(aq)]^{m+}$ and pure aquacomplex $[M(aq)]^{n+}$ at a ligand $L^{(n-m)+}$ concentration of 1 mol/kg (to be exact, activities, rather than concentrations, apply at such high concentrations). For convenience, usually the decadic logarithm $-\log k_{diss}$ is given. With concentrations denoted by swift brackets { }, the corresponding equation is:

$$K_{diss} = \{L\} \left\{\left[M\left(aq\right)\right]^{m+}\right\} / \left\{\left[ML\left(aq\right)\right]^{n+}\right\} \quad (2.1)$$

With Eq. 2.1 applying to a stationary situation (that of chemical equilibrium), it is likewise possible to interpret dynamic features – the catalytic behaviours of metal ions in the centers of metalloproteins – by this quantitative description of coordination chemistry. Then a transformation is considered during which some substrate is coordinated to undergo a reaction the product of which can be cleaved by a novel substrate because it is less strongly bound; this closure of a catalytic cycle will depend on both the transformation of ligating substrate and the peculiar metal ion which effects it. However, because the model relies on simple 1:1 complexes and their stabilities, it is crucial that ligand properties used in modelling are typical and specific for the very centers of metal–substrate interactions. This implies to focus on functional groups of both amino acid side chains of the apoprotein (or additional ligands such as porphyrine rings in haem or in methane synthetase) and those of the substrate.

2.2.3 A Method to Calculate Metal–Ligand Association Equilibria

Since the redox potential of some metal complex (couple) is an additive property (sum of electrochemical ligand parameters) for most cases (Lever 1990; Bursten 1982), with but small differences (≤0.1 V) for stereoisomers, it can be determined for some heteroligand-site chelators by interpolation between the "homogeneous" species, which means theat $E_L(L)$ for glycinate (–0.05 V) is the arithmetic mean between the values for oxalate (–0.17 V) and ethylene diamine (+0.08 V). Likewise the value for catecholate (which recently also was measured directly: Rocha et al. 2002 for aqueous medium) can be extrapolated by comparing $E_L(L)$ for phthalate (which in turn is represented by benzoate) and salicylate: glycinate formally can be constructed by "cutting" both oxalate (–0.17 V) and ethylene diamine (+0.08 V) "in half" (i.e., formally cleaving the C–C bond) to re-join the unlike ends. In an analogous way, values for other tri- or tetradentate heterodonor ligands such as iminodiacetate, ethylene diamine monoacetate or rhodotorulic acid (a diketopiperazine bis-hydroxamate produced by *Rhodotorula* yeast (Kaim and Schwederski 1993)) can be derived if c and x are also known for tri- or tetradentate ligands. With the latter tetradentate ligands, ligand sensitivity becomes very pronounced, with the "usual" positive sign with Cu(II) while those for Mg^{2+} and the trivalent Fe, Al and La ions are negative each. This is important given the role of such tetradentate ligands (hydroxamate siderophores, nicotian amine) in selective sequestration of Fe from soil; as nicotianamine is a oligomeric amino acid with correspondingly somewhat negative $E_L(L)$, it will prefer Fe and Mg to Cu. However, due to lack of data it was only possible to directly determine x_{4d} and c_{4d} for Mg and Cu(II) while the other values had to be obtained in the indirect manner based on the relationship by Irving and Rossotti (1956; ibid., and Martell et al. 1985):

$$Logk\left[\left(M'^{n+}\right)L\right] = a \times logK\left[\left(M^{Z+}\right)L\right] + c \quad (2.2)$$

where M′ and M are two different metal ions of (like or unlike) oxidation states, e.g. M = Fe(III) and M′ = Pu(IV) in a classical example (Duffield and Taylor 1987). The set of ligands (now with different denticities) used to derive the slope equation comprises fluoride (monodentate; 1d), glycinate and oxalate (2d), citrate and glyphosate (N-acetatomethylamine C-phosphonate; *Roundup*™; 3-dentate) and eventually hexadentate EDTA. To obtain meaningful data, one must make sure that high denticity ligands such as citrate, NTA or EDTA do indeed employ their full scale of binding sites in the given system

(which functionalized amino acids like glutamate or tryptophan mostly do not), e.g. by NMR or X-ray structure determination. Here, linear correlations are made between the logarithmic complex formation constants of Mg complexes of the above ligands and corresponding data (usually not available for all the ligands) for the ion M′. As c and x are known for both tetra- and hexadentate complexation of both Mg^{2+} and Cu^{2+}, values for some M′ and different hapticities can be derived using Eq. 2.2. In addition, this approach was used to control plausibilities of values directly determined with rather small sets of ligands with identical denticity; this was done, e.g. for La(III) and Fe(III). Values obtained by this control protocol are also collected in Table 2.3 (in brackets).

While the approach used in Section 2.2.4 requires to know values of $E_L(L)$ for bioligands (most data from Lever (1990)), it can also be "inverted" by rearranging Eq. 2.4 to describe the fractionation behavior of some biomass towards metal ions by defining some effective electrochemical ligand parameter from complex formation constants for different metal ions and a given ligand. The original $E_L(L)$ values were mainly obtained in solvents such as acetonitrile or dichloromethane, using (Ru) complex salts with organic counter-ions like $(TBA)^+$, but the available literature provides many data for biochemically relevant ligands like amino acids, nicotin amide (Lever 1990), other pyridines or oligophenol(-ate)s (Rocha et al. 2002) taken in aqueous media; additional ones were measured by us (Kollaske 2009; Fränzle and Kollaske 2008, to be published) for electroanalytic purposes. In addition, data for non-aqueous media can be directly correlated with those for hexacoordinate homoleptic Ru complexes in water (see below). Table 2.2 combines values from all the groups.

Using published Ru(II/III)redox potentials for $[RuL_6]$ or $[RuL'_3]$ systems (L = Cl^-, H_2O, NH_3, pyridine; L′= en, ox^{2-}, etc.) in aqueous solution and comparing those to the sums of $E_L(L)$, one obtains a smaller slope than in acetonitrile:

$$E_{measured:H_2O} = 0.907 \times \sum[E_L(L)] - 0.181\ [VNHE] \quad (2.3)$$

Such transformations render "aqueous" electrochemical ligand parameters like that for pyridine derivatives useful if the entire complex is known. Cyanide (i.e., the redox couple $[Ru(CN)_6]^{3-/4-}$) is an outlier from the above regression which can be expected given that cyanoligands of highly charged anions get protonated to become HNC ligands and then act as isocyanides, increasing the potential by some 0.35 V per protonated cyano ligand.

The list of "biorelevant" ligands includes both simple molecules and ions such as CO, N_2, acetate, sulfate or nitrate providing access to C, S or N sources by being

Table 2.2 Ligands involved in soil- and biochemistry. $E_L(L)$ denotes the electrochemical ligand parameter, the denticity is the number of binding sites which link metal ion centres and the ligand given in column 1. $E_L(L)$ values in brackets [ ] were calculated from complex stability constants with various metal ions in aqueous media rather than determined by electrochemical methods

Ligand	$E_L(L)$ (V)	Denticity n	Biologically relevant ligand (example)
α-Aminocarboxylate	−0.05	2	Glycinate peptide (N-terminal) 0.22 2 model:glycineamide
Porphyrinate	0.00	4	Haemin, chlorophyll
Sugar	[+0.11]	2 (?)	**Anion** of fructose (pKa = 12.3)
Nucleic acids	≈ −0.30 V	2–3	
Pyridine	+0.24	1	Vitamin B_6
Hydroxamate	−0.20	2	Rhodotorulic acid
Hydrogen carbonate	[−0.37]	2	
Carboxylate	ca. −0.21	1	Acetate, aspartate or glutamate residues (deprotonated)
Oxalate	−0.17	2	
Hydroxipolycarboxylate	[−0.26 … −0.13]	2 or 3	Malate, citrate
Phenolate	[−0.20]	1	Tyrosine residue, gallic and caffeic acids, humic acids
Chelating 2-acylphenolates	About −0.07 V	2	Salicylaldehyde anion, 2-acetylphenolate
Salicylate	[ca. +0.1]	2	
SO_4^{2-}	[−0.33]	1	
NO_3^-	−0.11	1 or 2	
Cl^-	−0.22	1	
Carboxamide	+0.03	1	Peptide, asparagine residue, urea
Carboxamidate	−0.38	1	

Bold: ligands which are known to be involved in metal transport in plants

coordinated as enzyme substrates, and also biomolecules which cover a considerable range of structural complexities (number and diversity of donor sites accessible to metal ions, of chiral sites, etc.), including amino acid side chains of proteins, the outward-directed polyphosphate backbone of nucleic acids, phosphorylated ("activated") sugars, metal-trapping peptides (glutathione, phytochelatins, siderophores, ferritins) and many others, thus providing correspondingly diverse coordination environments for metals which make their way into some piece of biomass. Accordingly within the same organism rather different complexation environments will arise, hence

1. Rather diverse metal ions – in all chemical respects including different oxidation states – can be fixed to biomass both effectively and selectively, whereafter these metal ions there can exert
2. A multitude of different and closely matching biocatalytic functions which also means that a new function to be established during evolution or ontogeny will not necessarily – not even as a rule – imply integration of yet another heteroelement (cp. the number of functional proteins in some highly organized creature – often many thousands – to that of available elements – less than 40 – and recall the rule of three functions!). Rather the rule of three functions will limit functionalization of heteroelements (cp. Chapter 4).

Properties which are relevant for bond stabilities are closely related to orbital energies, and the latter can be measured directly by electrochemical procedures. Hence the observed relationship between the electrochemical ligand parameter $E_L(L)$ and bond stability – even including hydrolytic stability rather than bond energies in the gas(eous) phase – might have been anticipated. This correlation is based upon the log-linear regression equation

$$-\log k_{diss} = x^{*}E_L(L) + c \qquad (2.4)$$

Equation 2.4 really is at the center of the entire way of argumentation. This equation and the c, x parameters derived to calculate it – as well as various permutations and applications – flatly provide the fundament of a quantitative understanding of the behaviour of metal ions (and, theoretically speaking; also other kinds of electrophiles) in both biomass and the embedding ecosystems. By this approach selective binding to certain metal ions and their corresponding clustering can be described and predicted (from the sign of x), with certain ligands distinguished by specific values of $E_L(L)$ (Table 2.2). This fact does influence both stationary (equilibrium) states and the activation of substrates and thus catalytic, in biochemistry enzymatic processes, the latter due to the fact that utmost catalytic chemistry be accomplished in cases of "intermediately" stable complexation, rather than very strong or feeble ones (Sabatier's principle).

To turn the way of argument upside down, consider data on redox potentials of ruthenium amino acid complexes, e.g. concerning *trans*-$[Ru(PPh_3)_2(glyc)_2]$ and similar alanine, leucine, phenylalanine or tyrosine complexes (Majumder and Bhattacharya 1999) and our own work (using proline, glycine, glutamate; Kollaske 2009; Fränzle and Kollaske 2009). The former authors report $Ru^{II/III}$ potentials (fully reversible) of 0.33–0.42 V vs SCE, but values were obtained in dimethyl sulfoxide or dichloromethane which precludes a direct comparison with $\Sigma E_L(L) = 0.56$ V ($E_L(L) = +0.38$ V for triphenylphosphine) from Lever's approach. The yellow complexes are stable, with both chlorides and one PPh_3 substituted by 2-aminocarboxylate from the educt in methanol or ethanol solutions which gives an idea of relative binding stabilities.

2.2.3.1 Complex Stability and the Electrochemical Ligand Parameter

As a conclusion, for metal-forming chemical elements pathways of transport through and strength/stability of binding to biomass, including possible bioaccumulation, are closely connected to their coordination properties. This defines the current endeavour: we are to express these coordination properties in a way (quantitatively) which allows to account for such abundance correlations (summed up in the BSE), biochemical functions and catalytic uses likewise. This requires a method of description which does not just address one "biochemical" ligand or another but encompasses the entire range of materials seen in biological materials and their interconversions.

Corresponding numbers thus must describe properties of some metal ion – be it essential, slightly or highly toxic – with respect to almost all kinds of donors. Some representation which borrows from data directly related to (semi-empirical) quantum chemistry (perturbation theory and ligand-field approaches,

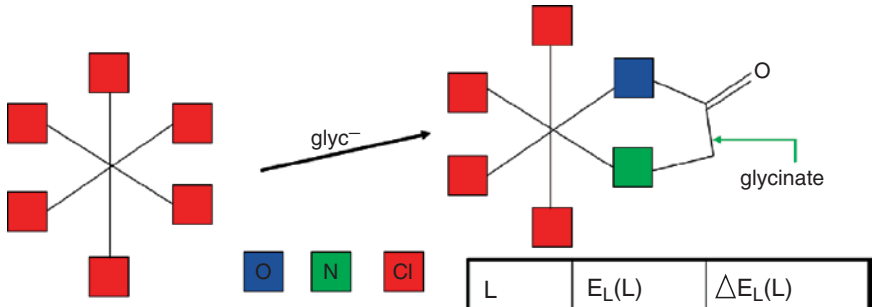
for example, measured by electrochemistry) will be most versatile provided it can link some easily measurable (or already measured) term to complex formation constants in a quantitative way referring to electronic properties of the complex thus formed – most versatile for understanding reasons of essentiality (which is neither caused nor implied by environmental abundances (cp. Al, Ti – very abundant yet completely non-essential so far – vs V, Mo, I which are rare but essential for many different living beings)).

This approach thus relies upon the following matters of fact:

- Complexes of one identical central ion with different ligands use to differ with respect to stability, however, stabilities of complexes containing ligands bearing similar or identical functional groups are very much similar, however, these implications of relative hydrolytic stabilities among, e.g. halido ligands F^-…I^- may vary with metal center. Water solubilities (net hydration enthalpies) of phenyl alanin(-ate) and glycinate or of ammonia and benzyl amine differ considerably, and so do those of their complexes. Hence the expected different lability towards hydrolytic cleavage of the complexes is almost cancelled, producing closely similar complex formation constants in these pairs of complexes for a multitude of metal ions each (e.g. Kiss et al. 1991; Sovago et al. 1993; Irving and Williams 1953; Irving and Rossotti 1956). As a result, binding stabilities just depend on the particular coordinating group, that is, carboxylate or an amino group while hydrophilic or hydrophobic behaviour (e.g. log k_{OW}) of the entire ligand molecule or anion is insignificant. Thus, the same "focussing" on the very binding (ligation) site may apply to very large ligands – that is, apoproteins or other macromolecules – also, allowing for a kausal interpretation of both the BSE and the observed patterns of (metal) essentialities.
- Upon exchange of a ligand by another there will be a change of both binding (hydrolytic) stability and of metal-centered orbital energy levels, causing the conspicuous changes of colour which are so typical for reactions in coordination compounds, classically accounted for by ligand field theory (Figgis and Hitchman 2000). Such energetic changes of highest occupied or lowest unoccupied orbitals influence the tendency of complexes to give away or take up electrons which in turn can be measured as a change of redox potentials which occurs due to exchange of some ligand.

Originally appropriate scales – that is, ligand-based electrochemical series describing redox potential changes – were developed in the Brighton-based workgroup of Chatt (starting in 1974, see especially Chatt et al. 1980a,b) and almost simultaneously by others (Sarapu and Fenske 1975; Bursten 1982). In 1990, Lever defined a kind of electrochemical series of ligands which refers to potential changes in the **Ru(II/III)** couple (solvent: acetonitrile). Accordingly the principal term was called the ligand electrochemical parameter $E_L(L)$. It (more precisely, its difference) gives the amount by which the redox potential in the Ru(II/III) couple changes upon some ligand replacement (when a ligand X gets substituted by Y). For multi- or oligodentate ligands this value has to be multiplied by denticity (hapticity) to obtain the change of potential which is actually seen (Lever 1990). The potential-changing effect is additive: replacing one carboxylate moiety by an aminomethyl donor site in a ligand system consisting of two carboxylates close to each other (oxalate, malonate turned into glycinate) produces an effect half as large as with replacing both sites (i.e. replacing oxalato ligands with ethylene diamine). The "absolute" scale also is derived from an assumption/observation (Chatt et al. 1980a; Bursten 1982; Lever 1990b; Rocha et al. 2002) of ligand additivity: for the famous redox couple $[Ru(bipy)_3]^{2+/3+}$ (bipy = 2,2′-bipyridyl), a complex which is used for photochemical water splitting, with six pyridine donor sites the standard potential is +1.56 V, for the respective Ru aquaion it is +0.24 V. Thus $E_L(L) = 1.56/6 = +0.26$ V for (half of) a 2,2′-bipyridyl ligand and $0.24/6 = +0.04$ V for water ($[Ru(H_2O)_6]^{2+/3+}$) as standard compound.

Like Lever did in 1990, aqueous redox potentials of other homoleptic ruthenium complexes – excluding ligand protonation like in $[Ru(CN)_6]^{4-/3-}$ – were used to determine solvent effects (see below). Let us give some example for calculation purposes only: $E_L(L)$ for a fluoroligand is +0.42 V, for chloride −0.24 V and for (bidentate) glycinate it is −0.05 V (Lever 1990). Thus, when $[RuF_6]^{3-}$ is treated by hydrochloric acid, replaving one fluoroligand to produce $[RuF_5Cl]^{3-}$ will increase the Ru(II/III) redox potential by 0.18 V whereas a reaction of $[RuF_6]^{3-}$ with glycinate to afford $[RuF_4(glyc)]^{2-}$ by replacing two fluorides will even enhance it by 0.74 V (Fig. 2.1).



Change of electrochemical ligand parameters (Lever 1990) and of redox potentials

L	$E_L(L)$	$\triangle E_L(L)$
F	– 0.42	
Cl	– 0.24	1F/1 Cl: + 0.18
glyc	– 0.05	2Cl/1glyc: + 0.38

Fig. 2.1 Complex ligand exchange reactions in the Ru(III) system (the d^5 ion Ru^{3+} is more reactive than the Ru(II) counterpart) and their electrochemical consequences. Recently, Ru(III) complexes with ligands known to be delivered by soil-dwelling organisms for sequestration of essential metal ions (e.g. malate, glycinate, hydroxamates, mixed ligands like caffeic acid) were prepared in our laboratory in order to measure the corresponding redox potentials, in addition using Lever's formula for recognizing the effect caused by replacing CH_3CN solvent with water (Kollaske 2009; Fränzle and Kollaske 2009)

This fact permits an (both kinetic and thermodynamic) analysis of reactions in coordination chemistry at redox-active central ions (Chatt et al. 1980a,b), including identification of the linkage isomer formed upon reactions with ambidentate ligands such as SCN^- (S/N), caffeic acid (phenolate + carboxylate or 1,2-diphenol; Fränzle 1992, this work) or dimethyl sulfoxide. Similar ligands like acetonitrile and benzonitrile or 4-methylpyridine and 4-cyanopyridine (each one bound via the pyridine nitrogen site), respectively, have almost identical values of $E_L(L)$, namely, +0.35 and +0.25 V. Therefore, $E_L(L)$ can be taken to identify identical groups binding metal ions (here, nitrile or pyridine moieties), allowing for an extrapolation to properties of similar ligands found in the natural environment. $E_L(L)$ and values derived using it in turn are suited not just to understand electrochemical processes at metal ions adhering to a biological matrix which then acts as a multiligand system but can also cover catalytic, including enzyme-based, processes.

The stationary case is given by the description shown below in Fig. 2.2. It is a sketch of the way by which complex formation constants are traced back to and correlated with $E_L(L)$ using the most simple approach of linear regression analysis (applications in Figs. 2.2 and 2.15). There are two terms in Eq. 2.4 one of which (c) describes an intrinsic binding stability independent of the kind of ligand while the other (x) is called ligand sensitivity as it depends on some ligand property, namely $E_L(L)$. The equation is:

$$-\log k_{diss} = x \times E_L(L) + c \qquad (2.4)$$

This Eq. 2.4 displayed by Fig. 2.2 and rearrangements thereof will return throughout this book as it is crucial to estimate binding properties, including dynamic features of metal ion catalysis, from the environment/substrate of some metal ion and a quantitative treatment of the properties of the latter.

Data on complex formation constants $-\log k_{diss}$ were taken from Irving and Williams (1953), Moeller et al. (1965), Izatt et al. (1971), Furia (1972), Kiss et al. (1991), and Mizerski (1997), plus values scattered elsewhere in the literature, while $E_L(L)$ values are derived from Lever (1990). This means that a potential (-shift) scale based on the Ru(II/III) redox couple is used rather than the older ones from the Chatt workgroup which draw upon Mo(0/I)-, Mn(I/II)- and similar low-valent couples (it should be pointed out that these concerning *trans*-$[Mo^{0/I}(dppe)_2(N_2)L]$ with L = Hal^- {F to I}, $ChCN^-$ {Ch = O, S or Se}, N_2, CO, PR_3, RCN, $CS(NH_2)_2$, etc. and dppe = 1,2-bis-diphenylphosphinoethane can be linked to Lever's scale by linear correlation with very high correlation coefficients (Fränzle 2005, unpublished)). The index nd (denticity n) corresponds to the

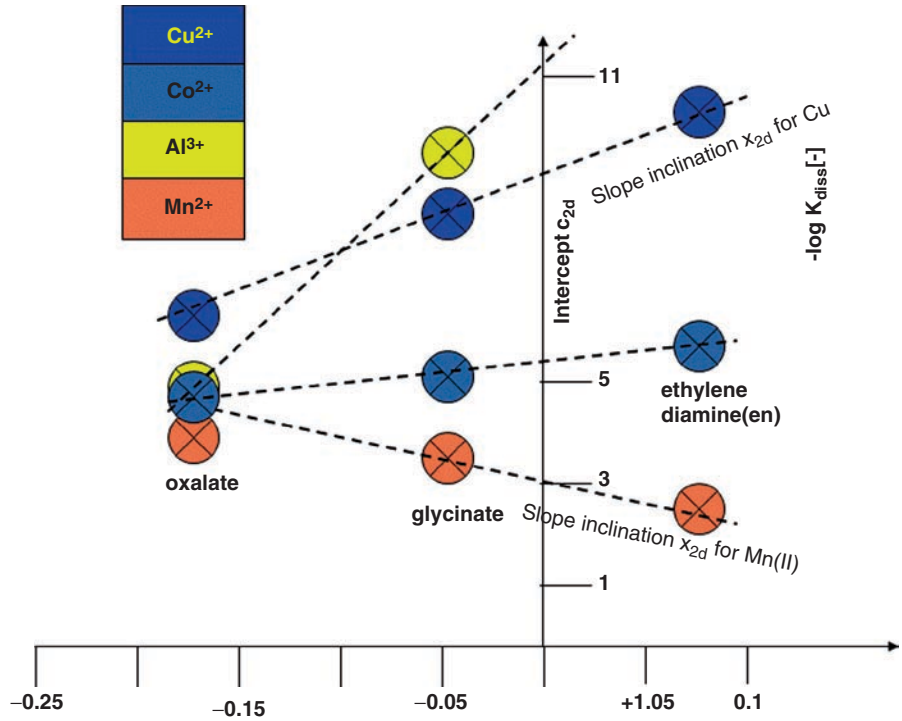


Fig. 2.2 Relationship between electrochemical ligand parameters of some bidentate ligands and their first complex formation constants with M = Al, Cu, Mn(II) and Co(II). Note the different sign of slope x_{2d}; that is, with Mn^{2+} complexes get the more stable the lower their $E_L(L)$ values are. Note that the sign of the slope (corresponding to x) differs (positive for three metal ions, negative with Mn^{2+}). This is presumably due to the different number of d-electrons: in d^5-Mn(II) complexes, unlike Cu(II) or Co(II), "spreading" of d-orbital energy levels will not cause decrease of redox potential but rather the opposite. With x expressing the influence of ligands on complex formation constants, c is independent of the ligand yet characteristic for the given ion and hence called intrinsic bond stability. It is identical with $-\log k_{diss}$ of ligands at $E_L(L) = 0$ V (perpendicular line). To obtain reliable data, a maximum of (mostly anionic) ligands and their complex formation constants (n >> 3) is employed. Although the ligands make use of different donor atoms (O, N, P, S, ...), the equation and c and x derived from holds generally except for Tl and Hg where there are different line equations depending on donor atom kinds (cp. Table 2.3). For additional explanations, see text

denticity of the ligands used for establishing the particular correlation (which must be constant). The first complexation constants are given for aqueous complex solutions at fairly physiological conditions (T = 25°C, I = 0.2 Mol/l, similar to blood or cell sap; 25°C are more realistic for plants in moderate climates than about 37°C for homoiothermic animals) are used. This is the equilibrium

$$k_{diss} = \{L\} \times \{[M(aq)]^{n+}/\{[ML(aq)]\}^{o+}\} \qquad (2.5)$$

Equation 2.4 combines two parameters which are typical for both the metal ion and the way the ligand is bound; the parameters are called (ligand) sensitivity x and intrinsic bond stability c. c is an acronym of **c**onstans, alluding to the fact that this part of bonding does not depend on the electrochemical ligand parameter while the sensitivity x does. X thus refers to the identity of the ligand (binding atom, its own chemical environment/charge (e.g. nitrite, amines, nitriles, pyridines, dinitrogen) and number of links to the metal center (hapticity η)). The slope x of this regression equation denotes the extent of sensivity observed in replacing one ligand by another. Once c and x are known, it becomes feasible to calculate/predict the behavior of certain metal ions in another ligand environment including biomasses or to calculate an effective ligand electrochemical parameter for biomass (or some well-defined ligand such as malate) by way of Eq. 2.4. While Lever's original list, though containing some number of biorelevant ligands like amino acids, certain heterocyclic compounds or imidazole, was derived from direct electrochemical measurements in the Ru(II/III) systems or extrapolated from redox reactions at Os or Cr complexes (Lever 1990), values given

in brackets in this work (e.g., –0.13 V for malate) were calculated from empirical complex formation data for a large number of metal ions, Eq. 2.4 and the corresponding c and x values for these metals.

When the concentrations of the metal ions in serum, cell sap or ambient water (aquatic organisms) are known, it can be determined whether some metal ion can become attached to a certain site, e.g. within a protein, at all. The end is to calculate and predict hydrolytic stabilities of complexes in biota or compartments of the biosphere, corresponding to equilibrium (2.4) between $[ML(aq)]^{o+}$ forming from L and the simple aquaion (aquacomplex) $[M(aq)]^{n+}$ and those two components. The first ligand exchange denotes the fact that several ligands with hapticities 1–3 can bind to a single metal ion. However, in cases of either low ligand concentrations or low stabilities the M-L-system will remain in this first step mainly; thus, many more complexation constants were determined for the first than for "higher" (subsequent) complexation steps. Both $-\log k_{diss}$ and the electrochemical ligand parameter $E_L(L)$ do barely depend on substituents on "external" sites (say, non-coordinating functional (nitro-, halo-, trimethylsilyl-, etc.) groups in aryls adjacent to the ligand site in, e.g. benzonitrile or phenolate or triarylphosphine ligands (cp. Fielder et al. 1995)), whereas different ligands – differing with respect to $E_L(L)$ – also differ in their complex stabilities: both complex formation constants and redox potentials change upon exchange of some ligand by another, say fluoride by chloride or CO by phosphines, with the reaction supported by the product complexes having larger stabilities.

A "colloquial" complex consists of one central ion and one or several ligands. For the most simple case (one central ion, one ligand other than water) the equilibrium constant according to Eq. 2.4 corresponds to $-\log k_{diss}$. This latter values refers to a relationship between metal ion and ligand whereas $E_L(L)$ describes the ligand alone. Accordingly, the property on this relationship between metal ion and ligand provides information on the other component of the system, that is, the metal ion, when $E_L(L)$ is known. For this purpose combinations (complexes) of one given metal ion with different ligands of identical hapticities (e.g. oxalate, glycinate end ethylene diamine) are investigated, taking the $E_L(L)$ values for the latter ligands (Fig. 2.2). c and x values thus obtained are specific for the metal ion and the given hapticity: e.g., for binding of monodentate ligands to REEs, rather similar c and x values are obtained while in bidentate systems, differences among these ions are quite considerable. Following an approach by Irving and Rossotti (1956), data for ligands of unlike denticities, e.g. F^-, CN^-, glycinate, iminodiacetate, citrate and EDTA, complex stabilities of which ligands correlate linearly with that for another central ion, may be used to determine additional c and x values for other metal ions by multiplying the regression data with c and x for well-studied metal ions (see Eq. 2.2). Thus, it is possible to determine c and x for some "exotic" metal ion – e.g. Be^{2+} or Sc^{3+} – even when only one single complex formation constant is given for a certain hapticity. Values thus obtained are given in brackets in Table 2.3.

Of course, the kind and "shape" of the statements on metal ion properties which are obtained depend somewhat on the mathematical way of analysis; here linear regression is selected because results are satisfying and, moreover, as $-\log k_{diss}$ is directly related (just proportional) to free reaction energies and the same approach is used in perturbation theory, there is formal agreement. Then c and x directly represent intrinsic and ligand-dependent binding properties in coordination chemistry of this metal ion. Whereas x can be either positive or (even highly) negative (e.g. in trivalent Dy, Nd, Ti or tetravalent Zr complexes) with chelating, bi- or multidentate ligands, c will be generally positive (even for Sr, Ba) for bidentate or higher bonding of di- or trications (cp. Table 2.3, Figs. 3.1 and 3.2). c_{2d} values extend from about –0.8 (Eu), +0.5 (Sr, Ba) to 9 (Cu^{2+}) and in trivalent ions from 2.0 (Nd) up to almost 12 (trivalent Fe, Al and Ga [1]) (Table 2.3). If $E_L(L)$ is close to zero, Eq. 2.4, that is,

$$-\log k_{diss} = x_{nd} \times [E_L(L)] + c_{nd} \qquad (2.4)$$

then simplifies to

$$-\log k_{diss} \approx c_{nd} \qquad (2.6)$$

regardless of denticity. Figure 2.2 shows how the relationship between electrochemical ligand parameter $E_L(L)$ and complex formation constant according to Eq. 2.4 operates in cases of several abundant metal ions and ligands.

[1] Apparently this difference of some 2–3 units is a simple charge effect, corresponding to the "electrostatic part" of M-L-binding, cp. the difference between c values of H, Tl, R_3Sn monocations vs. similar (isoelectronic, similar radii) dications (Be^{2+}, Pb^{2+}, R_2Sn^{2+}).

Table 2.3 Relationships and correlations among denticity of ligands and the parameters c and x for 54 Lewis acids which contain metal ions and the proton. Except for Rh(III), Co(III) and Fe(III)-porphyrines, the values correspond to the first complexation step. In biochemistry, two-dentate coordination (2d) and activation are of larger importance than the mono-dentate interaction; 1d accordingly denotes simple ligands such as NH_3 or fluoride. Values which were not obtained by direct correlation of multiple complex formation constants but rather using single constants and correlations with other metal ions are given in square brackets [ ]. There is no general relationship between the values of x_{2d} of REE ions and their redox properties, i.e. the capability to undergo oxidation (bold **element** symbol) or reduction (*element* symbol italicized) at least in some solvents (observed with **Ce**, **Pr**, *Nd*, *Sm*, *Eu*, **Tb**, **Dy**, *Ho*, *Tm*, *Yb*) although extent of stabilizations with O-donor ligands (polyphosphates, periodate) or fluoride should be largest for REE metals having particularly negative x_{2d}- or x_{1d}-values; the latter (but not the former) are almost identical with but a slight trend, concerning the +III oxidation state

Cation	c_{1d}	x_{1d}	c_{2d}	x_{2d}	c_{4d}	x_{4d}
H(I)	−6.52	−38.05				
Be(II)	−1.18	−16.66	[6.77]	[3.35]	[6.23]	[−8.36]
	[5.13]	[−1.87]				
Mg(II)	−0.1	−4.6	3.94	8.24	2.6	−20.6
Ca(II)			0.73	−10.98		
Sr(II)			0.55	−8.95		
Ba(II)			0.45	−8.02		
Al(III)	−0.78	−17.22	11.9	41	[7.07]	[−29.9]
Ga(III)	−0.24	−19.4	11.6	39		
Sn(II)	−3.84	−24.3				
Pb(II)	−0.50	−11.09				
Bi(III)	−1.07	−23.26				
Ti(III)			5.3	−63.7		
Zr(IV)			4.3	−46		
Hf(IV)			≥−2.7	≤−143		
V(II)	0.45	−12				
V(III)	0.5	−19				
VO^{2+}	[−0.51]	[−7.91]	6.3	−4.5	[4.13]	[−35.4]
			[6.44]	[14.17]		
VO_2^+			1.2	5.5		
Cr(III)	0.64	−15.4	[8.90]	[13.9]		
	[−0.01]	[−14.6]				
Mo(VI)			5.74	−3.78		
Mn(II)	0.3	−5.2	3.01	−5.32	6.7	−12.4
Fe(II)			4.20	6.05		
Fe(III)	−2.45	−22.42	11.26	21.39	[7.78]	[−58.36]
	[+0.13]	[−13.03]	[11.57]	[23.35]		
Fe(III) porphyrinate (haem)	+2.0	≈+5				
Co(II)	1.17	−5.3	5.48	3.93		
$[Co(CN)_5]^{2-}$	1.50	2.63				
Ni(II)			6.65	8.86		
Cu(II)	1.70	−8.0	9.04	21.84	19.1	26.5
Zn(II)	−0.29	−8.93	5.15	8.69	11.8	2.4
Cd(II)	0.70	−5.25	4.3	5.7		
Sc(III)	−1.19	−18.87				
Y(III)	–1	−11.6	4.71	−11.03		
La(III)[a]	−0.19	-9.48	3.02	−17.58	[5.31]	[−34.0]
	[–0.85]	[−7.60]				
Ce(III)	–0.56	−10.80	3.98	–11.94		
Ce(IV)	–0.84	−23.8				
Pr(III)	+0.12	−9.48	3.77	−11.43		
Nd(III)	–0.01	−9.87	1.99	-25.42		
Sm(III)	–0.47	–11.43	7.34	+7.58		
Eu(II)			[−11.3]	[–0.77]		

(continued)

Table 2.3 (continued)

Cation	c_{1d}	x_{1d}	c_{2d}	x_{2d}	c_{4d}	x_{4d}
Eu(III)	0.25	−11.09	3.2	+1.9		
Gd(III)	–0.69	−11.95	3.42	–19.63		
Tb(III)	–0.72	−12.29	7.79	+12.96		
Dy(III)	–0.73	−12.46	3.70	–24.24		
Ho(III)	–0.87	−12.61	3.47	–19.42		
Er(III)	–0.90	−12.70	4.02	–17.41		
Tm(III)	–0.93	−12.90				
Yb(III)	–0.97	−13.29	3.49	–20.33		
Lu(III)	–0.90	−12.55	4.14	–17.58		
Th(IV)	–0.29	−19.41				
UO_2^{2+}	–0.83	−14.35	9.15	+15.86		
Pu(IV)			[12.42]	[21.01]		
Am(III)			3.1	+1.0		

[a]The REE ions are well-suited for quantitative statements on bioindication for their being neither essential nor so toxic/thiophilic as to provoke exclusion reactions (i.e. phytochelatine induction) which likewise might change amounts and distributions beyond attention of chemical equilibrium. Concerning Ln^{3+}, the famous pronounced similarity of chemical behaviours is observed for monodentate binding only, with a trend towards weaker general complex formation (decreasing c) and increasing bias towards low-E_L(L) ligands (e.g., fluoride) from La ... Yb(III), whereas the differences are much larger with respect to bidentate, chelating ligands (Nd and Tb forming the extrema), without any consecutive "trend"

The vertical line in Fig. 2.2 corresponds to this kind of axis intercept which cuts in half the regression lines for the four different metal ions. The intersection of this line with that one displaying the effect of E_L(L) on complex stabilities gives c in any case, corresponding to stability constants of complexes of ligands which do not particularly stabilize or destabilize binding by $x_{nd} \times [E_L(L)]$ values far from zero. This is exactly the reason why c is called **intrinsic** complex stability of some metal ion. Such ligands for which $E_L(L) \approx 0$ V include (N-bound) nitrite, cyanide (both monodentate), diacetyldioximate (bidentate) or tetraphenylporphyrinate (tetradentate). x, which hence is called (ligand) **sensitivity** of the metal ion, denotes the size of influence of ligand variation, as mentioned before. Introducing c, x and E_L(L) into

$$-\log k_{diss} = x_{nd} \times [E_L(L)] + C_{nd} \qquad (2.4)$$

allows for calculating additional complex formation constants of this very cation. Sensitivity x can also bring about that there will be no more complex formation (in aqueous solution at least), namely if $x_{nd} \times [E_L(L)] << (-c)$.

This restricts the set of possible binding partners to some metal ion, pertinent to both stationary bonding and to catalytic cycles in which the substrate is coordinated to the metal center in the beginning. During biochemical transformation (biocatalysis), the substrate molecule/ion (ligand) will be converted into another species the functional groups of which differ from that of the original species and hence E_L(L) differs also. Using Eq. 2.4 allows to predict whether binding to the catalyzing metal ion is enhanced or weakened due to this transformation. In the former case it is likely to become permanently bound, with additional substrate incapable to remove the product; thus the catalytic cycle would break down by product inhibition. This kind of enzyme poisoning by product inhibition or irreversible "suicide substrate" binding corresponds to that of toxic substrates like cyanide, azide or monofluoroacetate.

Of course, qualitative (sign) statements on x can be derived from directions of spontaneously occurring reactions, with additional data from free reaction energies. In such cases, however, occupation of the last binding site is considered, rather than the first; on the other hand, more complicated fragments such as $[CpFe(CO)_2]^+$ can be described by their binding modes including (in this case, with both SCN^- and $SeCN^-$) the relative stabilities of linkage isomers: $[CpFe(CO)_2]^+$ does react with phosphines or alkenes, replacing CO, forms the more stable thiocyanato(S)linkage isomer (Burmeister 1968), and bound alkenes can be removed from this site with NaI in acetone; hence $x_{1d} < 0$ for $[CpFe(CO)_2]^+$ while CO removes alkene ligands in other (low-valent) organometallics such as $[Rh^I(CH_2{=}CHR)_2Cl_2]^-$ (corresponding to positive x_{1d}). Owing to the definition of the electrochemical ligand parameter (compensating for hapticity η) advanced by Lever (1990) which is used in Eq. 2.4 c uses to increase

with hapticity – not always in a steady manner (another representation of the "chelate effect") – for most metal ions whereas there are substantial variations of x vs η (division of the c and x values derived for bidentate ligands and many different metal ions (Table 2.3) shows just two cases where x_{2d} (bidentate) is twice as large as x_{1d} (monodentate), namely La(III) and Dy(III) but here, also, c_{1d} is far smaller than ½ c_{2d}).

2.2.4 How Does the Electrochemical Ligand Parameter Influence Real Versus Possible Hapticity of Some Polydentate Ligand?

One has to note that the first stage of complex formation which was used in these calculations – taking account of speciation with ligand concentrations common in biological materials and fluids also – corresponds to **ternary** species formed, that is, e.g. metal ion–glycinato–aqua complexes. In biochemistry, we deal with ternary and more complicated species also, e.g. considering metal ion + amino acid side chains + substrate assemblies hold together by complexation in some metalloenzyme or carrier species (for example, Cu^{2+} + imidazol [histidine residues] + $O_2^{(x-)}$ in haemocyanin) even though metal-centered chemical diversity does not go up to metal centered chirality. Since effects of chelating macrocycles are also known (cp. Fe^{3+} and Fe(III) porphyrines in Table 2.3), statements from this simple approach can be transferred to metal-ligand interactions in biochemistry directly. With multidentate binding partners such as porphyrines, corrines or deprotonated oligopeptides/cyclooligopeptides present, the fifth and sixth binding site in an octahedral complex also become relevant. Biorelevant ligands can be characterized using their interactions with metal ions also, and this is paramount for understanding metal takeup from soil or ambient water which former is mainly effected by delivering ligands to soil and reabsorbing the formed complexes by the same organs (roots or mycelia, respectively, often in combination when mykorrhiza is involved).

However, stereochemical effects cannot be inferred from the present data, i.e. whether the total hydrolytic stabilities of the isomeric square-planar complexes [Ni(ox)(en)] and *cis*- or *trans*-[Ni(glyc)$_2$] (ox = oxalate $C_2O_4^{2-}$, en = 1,2-diaminoethane ["ethylene diamine"], glyc = glycinate) are identical. Though there are some differences in electrochemical behaviour among stereoisomers (Bursten 1982, concerning [Mn(I/II) and Cr(0/I) polycarbonyl/isocyanide complexes], Bütje 1987 (dealing with hexakis-thiocyanato(S/N)- and halogenopentakis-selenocyanato(Se/N)osmates(III/IV))), ligand field spectroscopy does suggest that an additivity of complex formation constants as expected from Eq. 2.4 in this model system actually exists.

Like in the example of replacing two fluoro ligands with one glycinato ligand one has to multiply $E_L(L)$ for glycinate by hapticity two (also) to determine the change in redox potential quantitatively (Fig. 2.1). As noted before, x and c vary strongly between mono- and bidentate coordinations (except for Mn(II), Y(III) and Ce(III) where the x values are identical) and continue to do so for tri-, tetra- and hexadentate ligands, including even changes in sign of x (Zn(II), Mg(II)) (Table 2.3).

Using the now already familiar Eq. 2.4, made to be specific with respect to ligand denticity nd as

$$-\log k_{diss} = x_{nd} \times [E_L(L)] + c_{nd} \qquad (2.7)$$

x- and c-values were determined for many metal ions, plus the proton, certain organometal ions and neutral or cationic oxometal species like UO_2^{2+}, VO^{2+} and MoO_2^{2+}. Denticity is to denote the number of bonds between some metal ion and a single ligand; in cases of bridging coordination the denticity may be "distributed" to two, three or even more metal ions, especially with polymeric ligands like nucleic acids, poly(acrylamide) and the like. Notwithstanding this, what matters here is just the number of bonds to one given metal ion. These values for different denticities are given in Table 2.3.

These values correspond to the first binding of mono-, bi- or tetradentate ligands, however, if there are chelators in a metalloprotein such as porphyrines, or/and transport of neutral (O_2, NO) or anionic donor species by some metal-centered carrier is considered, the last ones are also important. Examples other than the above for the third (final) complexation of some chelator ligands with Fe(III) and Co(II), respectively (data from Mizerski 1997), are given in Table 2.4 below:

These parameters, intrinsic bond stability c and ligand sensitivity x referring to some certain hapticity, can now be applied for calculating and thus predicting complex formation constants for all the metal ions, complex fragments and organometal species in Table 2.3

Table 2.4 Last complex formation constants and corresponding modified c and x values for octahedral complexes of Fe(III) and Co(II) with chelating ligands

Metal ion	Ligand L	$E_L(L)$	$\Delta \log \beta_{2;3}$	$\log \beta_3$
Fe(III)	$acac^-$	−0.08	7.4	26.2
	$salicylate^{2-}$	+0.07	9.5	36.7
	ox^{2-}	−0.17	4.9	18.5
	$glyc^-$	−0.05	7.7	26
Co(II)	en	+0.08	4.2	14.8
	$glyc^-$	−0.05	2.4	11.1

Then, for Fe(III) $\Delta x'' = 18.70$ and $\Delta c'' = 8.45$ while $x'' = 74.76$ and $c'' = 31.15$ (both latter values are some three times larger than x_{2d} and c_{2d} for Fe(III)) whereas for Co(II) $\Delta x'' = 13.85$, $\Delta c'' = 3.1$, $x'' = 28.46$ and $c'' = 12.5$

provided the electrochemical ligand parameter is known for either the corresponding ligand or some analogous species; for example, the same values like for ammonia or ethylene diamine (en) also hold for higher saturated amines like diethylene triamine (see Lever 1990; Fielder et al. 1995). This is due to the definition of $E_L(L)$: the total effect is divided by the number of binding sites. Thus, e.g. a 2,2′-bipyridyl ligand can be treated like two isolated pyridines with respect to potential change in the prototypical Ru(II/III) system, except of course for "total" complex stability. The corresponding difference in c and x among different denticities thus implicitly also describes the "chelate effect".

2.2.4.1 Rules Which Can Account for Selective Metal–Ligand Interactions

There are two classical rules which predict what kind of ligand will be bound more tightly to a given metal ion (or atom). The older one, by Ahrland et al. (1958), forms different classes of metal ions according to the kinds of atoms they prefer to bind to – here considering only the atom directly adjacent to the metal, regardless of its own atomic environment besides the metal ion. For example, all nitrogen donors, such as nitrite, ammonia, pyridine or nitriles, are treated to be identical and opposed to P or As donors as another large group (which once again contains rather different ligands). In addition, there are the halide ligands F, Cl, Br and I and – not discussed there – carboxylates, phenolates, OH^- with their binding properties opposing those of S, Se or Te donors which may differ in local charge also for biological ligands (cysteine = thiolate, methionin = dialkylsulfide, etc.). With the first group, class A, there are more stable complexes with fluoride and the N (or O) donors than with iodide or some arsine, with class B binding preferences are the other way round (arsines, I^-, (Se donors) preferred). Though there are pretty many cases where this simple pattern does apply, it is obviously not capable to describe chemical transformations during which the atom bound to the metal does not change but only those to third atoms, e.g. the metal-ion-mediated oxidations of coordinated amines to nitriles or hydrolyses of the latter to afford carboxamides which thereafter are still ligated.

The other rule (Pearson 1963, 1987) distinguishes both metal ions and ligands with respect to their sensitivity against polarization ("hard" and "soft"). Hard centers combine high charges and small radii, making shift of charges difficult to achieve even in a strong electric field. Typical hard anions include oxide and fluoride, typical hard cations Be^{2+} or Al^{3+}. Generaly speaking, both hard and soft metal ions (acceptors, Lewis acids including molecules like SbF_5 (hard), $B(CH_3)_3$ (soft)) may react with rather different, either hard or soft anions or molecules (donors, Lewis bases) to produce salts, complexes, e.g. according to

$$SbF_5 + F^- \rightarrow SbF_6 - \text{(hard/hard)} \qquad (2.8)$$

or eventually metalloproteins, e.g. "borderline" Zn^{2+} or Cu^{2+} ions "accomodating" to a combination of one (very soft) thiolate and three (rather hard) imidazol (histidine residue) moieties. These and other conceivable combinations, however, differ in their stabilities. Now, Pearson's rule states that pairs of two hard *or* two soft chemical species are more stable (less soluble (in water), e.g. AgI vs AgF) than likewise conceivable combinations (which often also are produced in both synthetic and biochemistry) of hard, hardly polarizable metal ions (e.g. Al(III), Fe(III) or Ti(IV)) with soft ligands (bromide, thiolate, e.g. in cytochrome P_{450}) or vice versa (hydroxides, fluorides of soft cations such as Hg(II)).

Both acids and bases are described then as displaying pronounced or small "hardness", minimal or large polarizability, respectively (**h**ard and **s**oft **a**cids and **b**ases), the entire argument often is referred to as the HSAB approach. Notably, both very hard (Be^{2+}) and very soft (Tl^+, Hg^{2+}, polyorganotin- or –lead cations) acceptors are highly toxic; in addition, earlier work (Williams and Frausto Da Silva 1996) revealed that most essential metals are "borderline", corresponding to some transitional position in between typical hard and soft

acceptors. On the other hand, hardness values of biochemical substrates – including those to become directly bound to metal centers of metalloprotein enzymes – vary widely. Accordingly, the combinations found in biology should be scattered far on both sides of Pearson's optimum, with some advantage in versatility of binding and catalysis if such borderline ions are used. Notably, these combinations include abundant hard/soft combinations with macrocyclic or "soft-donor" protein systems: Mg(II) or $Fe^{III;IV\ (V)}$ combined with porphyrines (chlorophyll, haem and CP-450, respectively) or Zn-containing hydrolases (lactamases and phosphatases) using a Zn/imidazol/thiolate combination, with the lability thus created possibly useful in catalytic activity ("entatic effect").

A plot by Williams (1996) shows a special role for borderline Lewis acids in biochemistry. According to the HSAB approach they should be then capable of forming weak to moderately stable complexes with either hard or soft donors, producing complexes which are somewhat labile in any case whch is probably related to their catalytic functions.

Yet there is no relationship between "hardness" of some donor and its electrochemical ligand parameter, as can be seen from the sequence of $E_L(L)$ according to

$$OH^- < RS^- < F^- < Cl^-;\ I^-\ O\text{-aryl}^- \approx R\text{-}C\underline{O}\text{-}N(\underline{O})\text{-}R' < NO_3^- \gg \underline{S}CN^- < \underline{N}CS^- < RCH{=}NO^- < \underline{N}O_2^-;\ CN^- < [E_L(L) = -0.59 \ldots + 0.02V)$$

and it is thus impossible to analogize Pearson`s qualitative approach and the quantifying one given by Eqs. 2.4 and 2.6 in this work. The two-parameter approach is independent from both Pearson's HSAB approach and other ones which attribute one or two parameters to both donors and acceptors, e.g. that by Drago. A plot (mapping) of essentiality or other biochemical features vs the parameters c and x reveals the essential elements to form some cluster near the center of the distribution, that is, at intermediate values of both intrinsic bond stabilities and ligand sensitivities.

As shown before, it takes a quantitative method of complex stability prediction rather than some qualitative argument like HSAB (Pearson) or A/B patterns (Ahrland et al.) to give sense to the observed relationships among substrates/products and (biocatalytic) metal ions. It must thus be feasible to derive binding properties to a given metal ion directly from properties of substrates and products. Metals tend to be coordinated rather stably to plant biomasses; thus some larger part of them will not be dissolved in the xylem, phloem or cell juice, but "stick" to the solid part of biomass, forming (immobile) complexes there which often are retained in the roots already. Informations which in toto correspond to the Biological System of Elements thus are related to complex formation properties, that is, ligand properties of the (mainly plant) biomasses. There is selective binding, causing some metal ions to be far tighter bound – and thus far more enriched (higher bioconcentration factors BCF) – than others in a given organism. Regardless whether they are essential to this organism or not, such elements (of the first group) tend to be retained in substantial amounts and thus display fairly high BCF values. A typical case of this behavior is given by REEs in aquatic plants (Cowgill 1973; Nuclear Task Force 1996; Weltje 2003; Fränzle 2008) and iron bacteria (Fränzle and Noack 2008, unpublished) with BCFs between 5,000 and 3×10^5 and fresh water concentrations in the nmol/L to pmol/L ranges (Markert 1994b).

An understanding of the abundance correlations in the BSE and apparent abnormalities hence calls for quantifying these ligand properties while putting the behaviour of the metals into a numerical frame, too. This has to describe the extent and kind of selectivity in metal retention from the mixture which exists in the soil liquid or the broader environment (see, e.g. Still and Williams 1980 or Farago 1986 for this problem), besides determining the concentration of ligand sites (in μmol metal fixation capacity per g of DM in a given plant species).

Different assemblies, partition and distribution patterns of metal ions in different plant species are due to both differences in soil chemistry (not every kind of soil will support every plant species) and genetic and biochemical differences among plants (or among other organisms). Conceivably these differences correspond to unlike patterns of ligands in or delivered by biomass, including carboxylic and amino acids, alcaloids, etc. (the latter of which are crucial for biochemical taxonomy also, e.g. with Ranunculaceae). Though (hydroxi)oligocarboxylates delivered by plant roots, such as citrate, oxalate and malate, are strongly involved in fractionation of heavy metals between rhizosphere and root tissues, this fractionation cannot be completely attributed to the above complexation properties (Still and Williams 1980; Farago 1986). Thus, complex formation constants of citrato-, malato- or oxalatometallates will not fully explain metal resorption and transport in vascular plants. In their model of transport, Clemens

et al. (2002) invoke "other chelators" located in between the upper parts of the rhizosphere and the shoot, obviously involved in fractionation by selective transport, but for now their chemical identity is unknown. Therefore, once again, a theoretically supported chemical formalism is required which allows to unravel the chemical conditions when the fractionation is known. In the best case, such a formalism should be capable to identify active ligating positions in biomass. For this purpose, an empirical parameter (which can be measured) must be defined in a way to produce a direct link between some characteristical properties akin to a metal ion and and the complex formation constants. Whereas "normal" quantum-chemical calculations just produce information on one certain compound rather than describe an entire "class" of compounds (Primas and Müller-Herold 1984), such empirical parameters *may* (cp. e.g. Nieboer and McBryde 1973 for some ligand-specific definition of binding properties even in sets of very similar ligands) refer to entire classes of ligands or, more precisely, to the corresponding functional groups along which complexation takes place.

Because kinetics of complex hydrolysis is typical for a given metal ion (Tobe 1976; Jordan 1994), it would suffice to obtain some relation similar – and perhaps closely related – to the Hammett or Taft equations which gives rates for "admission" of some ligand to replace water to calculate the ratio of forward and backward (hydrolytic) reaction rates and thus the state of equilibrium. This is due to backward (hydrolytic) reaction rates being fairly constant for a given metal ion. There is work on relationships between the electrochemical ligand parameter and Hammett's substituent constants, both adressing substituents "as such" anionic forms of which can also act as ligands (H, Cl, $SiPh_3$, CN, NO_2, $-NH_3^+$, etc.) and substituent-bearing aryl groups in thiolates, phosphines, arsines, etc. (Fielder et al. 1995). The first yields a strong correlation with Hammett's substituent term, the effects in the second case are small which by Eq. 2.4 then also holds for complex formation constants. However, quantitative coordination chemistry provided much more data on equilibria directly (starting with the classical papers by Bjerrum and coworkers (about 1950)) than works dealing with separate kinetics of forward and backward (solvolytic) reaction rates (e.g. Merbach 1982).

However, there are ways to determine energetic levels in some molecule or complex, including changes which occur by replacing some substituent (or in complexes, some ligand) in a direct way, by removing electrons from the HOMOs either by electromagnetic radiation (photoelectron spectroscopy) or at an anode (electrochemistry), with wavelength, electron excess energies or redox potentials providing the information on energy levels. In organic electrochemistry redox potentials were correlated with excitation wavelengths producing triplet states of PAHs rather long ago (e.g. Lund 1957; Heilbronner and Bock 1978), and there is a relationship (of anodic potentials and reaction rates towards oxidants such as OH radical) to Hammett`s substituent constant once again (Fränzle 2000; unpublished work). A direct electrochemical probe into substituent (that is, ligand) effects in coordination complexes is afforded by the "electrochemical ligand series" (Lever 1990), with data available for literally hundreds of ligands, including many ones pertinent to biochemistry.

This state of affairs, with lots of empirical data on some parameter which can be easily measured at hand, combining with a relationship to some general feature of functional groups (donor sites of ligands), prompted this author to combine Bjerrum's approach to that of Lever. This means deriving an equation (empirical once again rather than based on ab-initio calaculations) which links complex formation equilibria to electrochemical measurements on ligand effects (photoelectron spectroscopy does not provide similarly exact values on substituent effects whereas metal NMR or Mössbauer data (the latter available for few nuclei such as ^{57}Fe, ^{119}Sn, ^{191}Ir, ^{192}Pt, ^{197}Au or ^{45}Sc only) give information on electron densities and energies next to the metal atom nucleus rather than to binding orbitals).

The same as for a stationary state of complexation holds for activation by coordination, fixing and activating the substrate of an enzyme; now there are second-order effects brought about by the other ligands attached to the metal ion (e.g., some apoprotein). For redox-active ions, these ligands also contribute to the overall actual redox potential of the metalloprotein. This makes us expecting some relationship between the ability to fix and activate certain substrates and the total redox potential of the complex, linking the latter to specific (often selective) catalytic abilities. In bioinorganic chemistry, relationships between the redox potentials of enzymes containing Fe or Cu and kinds and rates of their specific biocatalytic activities are well established (Kaim and Schwederski 1993; Williams and Frausto Da Silva 1996). This is also observed in "model" compounds, e.g. with the capability to protonate and reduce (eventually

yielding and releasing either hydrazine or ammonia) coordinated dinitrogen (Chatt et al. 1980a): Mo and W complexes having excessively high M(0/I) potentials (e.g. L^6 = CO, $P(OR)_3$) will not replace other ligands (water, Cl^-, solvent molecules such as THF) with N_2 at all, while ongoing protonation, producing $-NNH_2$ moieties first and then free N^{red} species (hydrazine or ammonia), is observed only at ever lower potentials for the Mo(0/I) couple which are obtained by introducing appropriate "sixth" ligands (four sites are occupied by two diphosphine ligands, the fifth by N_2) like imidazole, Hal^-, SCN^-. Note that in native nitrogenase N_2 complexation and reduction do **not** take place at Mo (or V, W) ion sites, but at Fe (with the dinitrogen ligand bridging two Fe ions; Eady 2003). Accordingly, the influence of redox potentials – and thus of (sums of) electrochemical ligand parameters – does not start with activation of some substrate but already controls coordination to the activating center of some metalloenzyme or other kind of complex only but already controls fixation to this center; hence the relationship given by Eq. 2.4 or some equivalent of it is not restricted to the first step of complexation but likewise applies to its final steps, with metal ions attached to the apoprotein "lattice" by just two or three donor sites as a rule. Of course, the redox potential depends on the sum $\Sigma E_L(L)$. Substrate specifity in turn depends on the coordinative environment of the central ions of the metalloproteins, including chiral information which is due to amino acid side chains or cofactors (say, porphyrine rings) bond there.

The capability to induce and catalyze some reaction once again depends on the corresponding $-\log k_{diss}$ (see above). This makes it feasible to derive parameters (namely, c and x) which describe binding properties of some central metal ion (or other electrophile or neutral species) by just $E_L(L)$ of the reaction partner – be the reaction stoichiometric (formation of a stable complex) or catalytic (with a subsequent transformation of the ligand and loss of product) – and these terms.

There are several reasons why in many polydentate ligands only some part of the donor functions is employed in binding one metal ion, e.g.

(a) Rigid linear structure of the ligand with donor sites at either terminus, favoring bridging but precluding chelation (e.g. with SCN^-, $SeCN^-$, aminoacetonitrile, pyridine-4-carboxamide)
(b) Sterical hindrance and of course
(c) Very unlike complex formation constants with the various donor sites, given that x_{2d} differs from zero considerably, for example, with the formal amino acid ligand analogs P,P-diphenylphosphinoacetate or As,As-diphenylarsinoacetate[2], finally
(d) The number of donor sites provided by the ligand may be >6 (or >11 when REE centers are concerned), e.g. in potentially octadonating DTPA

Several of these problems also refer to proteins if these accomodate metal ions. When a metalloprotein forms, there thus will be the issues of the number and location/properties of the "actual" complexation sites and whether there will be linkage isomerism (Burmeister 1968; Bütje 1987; Fränzle 1992), the latter possibly controlling pathway and kind of a catalytic transformation: after the first case of linkage isomerism was established concerning nitrite NO_2^- ligands back in 1893, the very next to follow were biomolecules, namely Co(III) complexes of cysteinate activating two different pairs of donor atoms in this also rather small ligand molecule (N + S or N + O, respectively; Balahura and Lewis 1976); more recently, the interest in coordination modes of metal ions, including toxic ones, to nucleic acids grew (Sigel and Kapinos et al. 2001), especially with respect to the mode of action of cytostatic agents (Fränzle and Markert 2003). As for the validity of c- and x-parameters, be they directly or indirectly obtained, linkage isomers and corresponding ligands (nitrite, thiocyanate (see the notice in Table 2.3 concerning c_{1d}, x_{1d} of Fe(III)), sulfoxides (due to formation of a series of linkage isomers, the redox potential for the $[Ru(DMSO)_6]^{2+/3+}$ couple can vary by more than 800 mV in the same solvent)) must be avoided unless the site of coordination is precisely known since occurrence of linkage isomers usually means that thermodynamic equilibrium was not reached in bonding. In the classical Co(III) complexes, x is close to zero, supporting formation of such isomers.

Metal ions in metalloproteins tend to be rather feebly bound (Williams 1986), except for Cu(II) and few others, using just two to three coordination sites which link them to amino acid side chains of the protein. This fact has various ramifications for conditions of essentiality in a reproducing system. Figures 3.1 and 3.2 show some correlation between certain values of c and x (which are coupled to each other as long as the oxidation state is kept constant)

[2]Conversely, either chelating or linkage-isomeric behavior in a ligand with very different $E_L(L)$ of the possible donor sites points to $x_{1d} \approx 0$, e.g. with acrylate ion complexes (either behaving as an olefin π-ligand [side-on-bound] or as a carboxylate) of Pt(II).

on one hand and essentiality or other biochemical features on the other. Of course, such correlations can be no more valid (precise) than the determination of c and x itself, and hence ambidentate ligands or unsettled coordination sites must be avoided once again. In addition, hapticity also is an issue as it is not straightforwardly possible to infer c and x values for other denticities from those for mono- and bidentate behavior: for nd = 1–6, even the sign of x may vary either periodically or irregularly (whereas c does steadily increase), with a concomitant (a-)periodic change in metal preference considering that the number of actual binding sites is far smaller than those theoretically offered by the amino acid side chains. The actual binding behavior points to usually bidentate behavior of the protein macroligand which thus is assumed in Fig. 3.1. Figure 3.1 provides some mapping of this kind; however the two inquiries into the "actual" number of donor sites which link the apoprotein to the metal ion and into linkage isomerism are closely related: as there are many more potential binding sites in the protein (often occupied by several identical or different metal ions), the real one just represents one of many conceivable linkage isomers, including permutation of binding sites among different metal ions in, say, a Zn/Mg enzyme, and the relative stabilities can be inferred from Eq. 2.4 once again because electrochemical ligand parameters for the sites inside the protein are also known. For those cases when either metals or binding sites may vary in active enzymes, these yet represent but a tiny part of the possible arrays, thus of possible linkage isomers. Besides spatial change, there also was and still is temporal change: increase of structural complexity take place both during chemical evolution (Chapter 4) to form multiligand-site "metallizable" polymers for the first time and during ontogeny or development of some embryo. Unlike with complexing some molecule or anion from a solvent or binding the latter, a protein might bring about some rigidity after folding which may not permit establishment of the "optimal" M-L bond distances and L-M-L′-dihedral angles around the metal center which may be the reason for "abnormal" optical and NMR spectra associated with the metal ion, plus an increased or modified catalytic activity ("entatic effect", Vallee and Williams 1968). However, these deviations usually vanish upon addition of the substrate or some other species which gets coordinated which suggests that the usual hexa- or tetracoordinate ligation polyhedra are formed then, with no more structural distortion even though

(a) The rigidity of the protein matrix still exists,
(b) Steric interactions should even be increased by inserting one more ligand spatially more demanding than Cl^- or water.
(c) Access often does and can occur only along a trajectory which is dictated by the catalytic cleft.

Yet, given the large spectroscopic (UV/VIS) and chemical (equilibrium state, e.g. O_2 affinities in distorted, congested Co or Ni ("lacunar") complexes) effects of distortion also described by the angular overlap approach this result is most telling: the substrate-loaded metalloproteins apparently are very close to equilibrium configurations, strongly changing relative affinities of different halide and pseudohalide ligands to protein-fixed Zn centers.

As a most simple model for such a development, some potentially tetradentate organic oligomer is considered which may switch between two- and fourdentate complexation. Given the differences between x_{2d}, x_{4d} and c_{2d}, c_{4d}, respectively, one can figure out for which ranges of (average) electrochemical ligand parameters some metal ion will stop using all four donor sites rather to bind by just two of them. This is not about linkage isomerism alone: this kind of "switch" also implies that one or more binding sites are "left open" for activation of substrates. Then, the values of $E_L(L)_{crit}$ given in column eight (Table. 2.5 below) represent some optimum condition for activation by that metal, fulfilling Sabatier's principle, with $E_L(L)_{crit}$ representing an average value if different donor sites are involved: if the actual average of donor site $E_L(L)$ values is close to the calculated value, catalytic turnover will be largest with the corresponding central ion (Table 2.5), or, vice versa, among the (very) large set of linkage isomers possibly forming with some polymer, efficient enzymes are those where the identity of the ion is close to the "equilibrium" value or – vice versa – a given ion can behave as a catalytic center if and only (?) if it accommodates a suitable position. What does this imply with respect to the "map of essentiality" (Figs. 3.1 and 3.2, respectively)? The values given below give proof that – out of a set of all essential, non-essential and highly toxic ions – $E_L(L)_{crit}$ may vary widely, even beyond the range of realistic values whereas some non-essential or detrimental ones (Be, Al) may agree with essential ions like Mg, Fe.

Table 2.5 c and x values and relative stabilities of bi- vs tetradentate binding of some ligand. The "critical" values correspond to some sum of ligands, owing to the definition of the electrochemical ligand parameter

Metal ion	c_{2d}	c_{4d}	$c_{4d} - c_{2d}$	x_{2d}	x_{4d}	$x_{4d} - x_{2d}$	$E_L(L)_{[crit]}$	$-\log k_{diss}$
Be(II)	(6.77)	6.23	−0.54	(3.35)	−8.36	−11.71	−0.04	6.6
Mg(II)	3.94	2.6	−1.34	8.24	−20.6	−28.84	−0.05	3.6
Al(III)	11.9	7.07	4.83	41	−29.9	−70.9	−0.07	9.2
La(III)	3.02	5.31	2.29	−17.58	−34.0	−16.42	+0.14	0.55
Mn(II)	3.01	6.7	3.69	−5.32	−12.4	−7.08	+0.52[a]	0.24[a]
Fe(III)	11.26	7.78	−3.48	21.39	−58.36	−79.77	−0.04	10
Cu(II)	9.04	19.1	10.06	21.84	26.5	4.66	−2.16[b]	[b]
Zn(II)	5.15	11.8	6.75	8.69	2.4	−6.29	+1.07[c]	14.4[c]

[a]A theoretical value, cannot be realized with anionic ligands. $E_L(L)$ being always (considerably) smaller, maximally tetradentate ligands such as nitrilotriacetate will indeed bind to Mn^{2+} in a tetradentate mode with $-\log k_{diss} > 0.24$, notwithstanding redox-induced lability. Besides, such large concentrations of multidentate ligands (≥0.6 mol/kg) are outright unrealistic in vivo

[b]Unrealistic situation, since tetradentate binding to Cu(II) will be always stronger as x_{4d} does not differ too much from x_{2d} for this ion

[c]Cp. Mn(II) for (reasons of) the impossibility to realize the critical value of $E_L(L)$

This is due to the fact that this particular equilibrium does but describe some very special case: the activation of a bidentate substrate which undergoes activation by direct complexation to the metal ion. In other systems, the critical values will be different.

These values of −0.05 V for Mg (which corresponds to $\Sigma E_L(L_1, \ldots, L_4) = -0.20$ V, e.g. a his_2asp_2 surrounding) or +0.52 V for Mn(II) or +1.07 V for Zn(II) are generally larger than for the principal donor sites of proteins (other than imidazol [histidine], indole [tryptophan]) and of nucleic acids, hence bidentate binding is favored for these polymeric ligands also although there are really lots of additional donor functions around the metal ion; the substrate yet can be added and activated as it is no part of the protein backbone but a separate ligand. Tetradentate complexation, on the other hand, is but a transient phenomenon, then, effectively disallowing linkage isomerism. Recall that $E_L(L)$ generally depends just on the kind of donor sites and atoms (plus the anionic charge density) but not on the structure or total size of the ligand; hence for a polymer the same values will result as for simple similar ligands unless there is pronounced distortion of the coordination polyhedron (cp. data from Vallee and Williams 1968). For chelate complexes of different denticities, x can either be positive or negative (see Tables 2.3 and 2.5 above) – for many different metal ions – which will increase "adaptive" flexibility; for bidentate behavior, three of the essential elements (Mo, Mn(II) and Ca which latter, however, rarely exerts catalytic functions) have negative x_{2d}.

Now we will extend this approach to equilibria between four- and sixfold coordination of the same, coherent large ligand, just for comparison. Data for hexadentate coordination are available with both natural (nicotianamine) and synthetic (EDTA) amino acids, and hydroxamate siderophores (coprogen, desferrioxamin; Enyedy et al. 2004) and thus are related to biorelevant systems directly. The line of reasoning is the same: activation of donating substrates (e.g. carboxylates, sulfite, phenolates, etc.) is most efficient for cases of dynamic equilibrium between tetra- and hexadentate linking with the polymer matrix. Here, the implicit assumption concerning the starting step that molecular recognition of the substrate which makes its way into the catalytic cleft does not necessarily bring about coordinative binding of this substrate to the matrix; rather electrostatic (cp. Lipscomb 1982) or van-der-Waals (e.g. between lipophilic substrates and side chains of aromatic or large aliphatic amino acids) interactions will do. Electrostatic interactions then tend to be stronger than average activation energies without any catalyst. Given this and a labile equilibrium between four- and sixfold metal-protein binding, with water and other simple species such as chloride occupying the two remaining positions, exchange kinetics of the latter ligands, particularly water, determine kinetics of the catalytic processes. In Table 2.6, there are corresponding values for essential elements Mn, Fe, Cu and Zn, plus Al.

Here, critical electrochemical ligand parameters are much closer to each other and to the realistic range of EL(L) than for the 2/4-equilibrium discussed before; in addition, the complex formation constants

Table 2.6 Equilibrium conditions for tetra- and hexadentate binding of some macro- or mesomolecular ligand anion with ≥6 donor sites

M	c_{4d}	c_{6d}	Δc	x_{4d}	x_{6d}	Δx	$E_L(L)_{crit.}$	$-\log k_{diss}$ (equilibrium)
Mn(II)	6.7	5.1	−1.6	−12.4	−74.0	−61.6	−0.03	7.1
Fe(III)	7.78	28.03	20.25	−58.36	31.92	90.28	−0.225	20.9
Cu(II)	19.1	16.13	−2.97	26.5	−26.63	−53.13	−0.06	14.6
Zn(II)	11.8	12.42	+0.62	2.4	−35.47	−37.87	−0.02	11.4
Al(III)	7.07	22.00	14.93	−29.9	50.84	80.74	−0.185	12.6

for Mn(II), Cu(II) and Zn metalloproteins are rather close to biochemical reality (cp. Williams and Frausto Da Silva 1996). Given $E_L(L)$ for carboxylates, phenolates, phosphorylated compounds (ribozymes, abzymes!) or hydroxamates, and the fact that Fe(III) also is involved in many metalloproteins, the value of $E_L(L)_{crit}$ for Al obviously is not at odds with a possible biocatalytic role for this highly abundant ion; the actual problems will be discussed lateron. The "window of essentiality" is constructed by metal ion – rather than ligand properties akin to proteins, peptides or NAs. Yet, after hydrolytic processes, amino acids, peptides, methionine will be more strongly bound while phenolates should be readily cleaved and expelled by the matrix returning to hexacoordination of the metal center. Conversely, Mn-, Zn- or probably also Mg-based phosphatases are well-suited for complexation of the oligophosphate precursor replacing two M-protein bonds as the $E_L(L)$ of organophosphates are far lower than that "critical" values. Weak ligands like water or chloride will be removed by the substrates as readily. Concerning Al, there would be another problem even though it does catalyze certain biorelevant transformations such as redox transamination (interconversion of 2-oxo- and α-amino acids): the hydrolysis of (oligo-)phosphorylated species presumably would be catalyzed, too, but then, owing to the tiny solubility of $AlPO_4$, the enzyme would destroy itself. Like for the bidentate ligands for which the original Irving–Williams series was derived (Irving and Williams 1953), the ligand sensitivity increases with hexadentate ligands as Mn(II) << Zn(II) < Cu(II) < Fe(III) < Al(III) (with bidentate ligands x_{2d} is almost identical for Cu(II) and Fe(III)).

In addition, potentially multidentate ligands like proteins (theoretically, nd > 100) will accomodate several identical or different metal ions during metallization, and there are cases in which differently highly metal-ion-loaded metalloproteins will fulfill the same or very similar catalytic functions, e.g. in phosphatases and nucleotidyl-transfer enzymes (Yang 2008). There is a strange effect: if there is only one position occupied by a metal ion, many different metal ions will accomplish the function, among them divalent Mg, Ca, Mn, Zn, Cu, Cd, Co and Ni whereas the "two-metal-version" will only display biocatalysis when both positions are occupied by Mg^{2+} (Yang 2008) with the oligophosphate substrate replacing one pair of links of an aspartate side chain hitherto bound to Mg while in other metalloproteins heterogeneous loading is required for function, e.g. with Mn/Cu or Cu/Zn peroxidases (Kaim and Schwederski 1993; Williams 1986; Williams and Frausto Da Silva 1996).

Intrinsic bond stability c and ligand sensitivity x jointly characterize the features of a metal ion with respect to coordination chemistry. For this reason, the list given above can be corroborated by an approach which directly refers to coordination chemistries of the metals – whether they behave as sequestration or transport agents ("static" interaction) or take part in biocatalysis ("dynamic" interaction). As a general rule, most chemical reactions are reversible on a microscopic scale, whereas no catalyst will ever shift the position of chemical equilibrium, i.e. the relative rates of "forward" and "backward" reactions, but also enhance kinetics of the latter. Accordingly some enzymes will enhance rates of reactions yielding "opposite" products likewise, e.g. acetaldehyde oxidoreductase producing either acetate or ethanol from acetaldehyde, or can be used in either direction, e.g. formate dehydrogenase obtained from clostridia. *In vivo*, formate dehydrogenase oxidizes the organic anion but in vitro also efficiently accomplishes CO_2 fixation and reduction making $HCOO^-$ if immobilized at, e.g. a p-InP photoelectrode, allowing for chemical storage of light energy at remarkable ≈11% yield; alcohol dehydrogenase also is involved in acetaldehyde reduction to produce ethanol in yeast, etc. Nevertheless rapid turnover requires the product to be less tightly coordinated to the metal center (if there is direct interaction indeed) than the

original substrate. Perfect reversibility thus implies either

(a) Lack of direct interaction between metal center and substrate
(b) High similarity of binding constants in product and educt (substrate) or
(c) Additional processes which rapidly remove the corresponding product from the enzyme, such as coupled secondary reactions

If none of the above cases is given, e.g. some substrate is taken directly from the environment or some product delivered to it with a transient direct metal-intermediate interaction, the both directions are no longer equivalent, and thus, with the forward reaction, say reduction of dinitrogen to ammonia being promoted by some metal center having positive x, the backward reaction will be accomplished by some metal having negative x, or vice versa, which will be favorable, depends on the sign of change of electrochemical ligand parameter during the transformation and possibly on a change of hapticity (note that there are very few cases in which x_{2d} really is twice as large as x_{1d}). This fact often necessitates to determine $E_L(L)$ by calculation (Eqs. 3.2 and 3.3) even though literally hundreds of values were determined before (Lever 1990 and additional works).

Equation 2.4 can be rearranged in order to calculate an electrochemical ligand parameter from complex stability data provided both hapticity of ligand and c and x of the central ion are known. The results are compiled in Table 2.2, rearranging Eq. 2.4 in modified form (2.7) as to obtain $E_L(L)$:

$$-\log k_{diss} = x_{nd} \times [E_L(L)] + c_{nd} \qquad (2.7)$$

gives

$$x_{nd} \times [E_L(L)] = - c_{nd} - \log k_{diss} \qquad (2.9)$$

and thus

$$E_L(L) = \{- c_{nd} - \log k_{diss}\}/x_{nd} \qquad (2.10)$$

This was done not only concerning fairly simple oligodentate ligands of biochemical significance such as glycerolaldehyde-3-phosphate or some proteins for which the ligand environment of the metal center is precisely known but it is feasible to extend this approach to mixtures of ligands (without or among other compounds) and even to biomass (a certain organ of some species). This implies Eq. 2.10 to apply to such cases where there is no well-defined ligand or even a mixture of ligands with identical donor groups. Then, a comparison of complex formation constants which are tackled in the colloquial manner affords a numerical value which corresponds to an *average* fractionation capacity of that mixture or biomass sample with respect to metal ions. Yet the scope is broader: toxicity issues in comparison of various heavy metals or other biochemical properties such as biocatalytic efficiency can be addressed in the same way as simple accumulation or fractionation (including the fact that fractionation may be "amplified" relative to differences of complex stabilities by multiple speciation events or by transmembrane transports).

The "theoretical" value of an effective $E_L(L)$ obtained using Eq. 2.10 in addition can be used to obtain some hints on identities of coordination sites provided analytical data, values of Brønsted acidities or basicities are at hand for such a polymer. In toxicology, these coordination sites are tantamount to sites of action, e.g. "soft" heavy metal ions can bind to thiolate residues and thus block them. If this were the only mode of (relevant metal ion-protein inter-) action, the electrochemical ligand parameter for relative toxicities would be −0.55 V, i.e. that of the thiolatoligand. However, the agents which serve to store and trap (excess amounts) of "soft" heavy metal ions are a little bit more subtle in their structures, e.g. phytochelatin displays an alternating array of thiolato- and carboxylato functions. Experiments (Dorčak and Kręcel 2003) on variants of native phytochelatin showed this highly "ordered" arrangement to be best-suited for trapping of Cd^{2+} ions. In mixed-center multidentate ligands the overall $E_L(L)$ is an average over those of the individual sites (e.g. that of glycinate is the arithmetic mean of the values which hold for (a) oxalate and (b) ethylene diamine); likewise in phytochelatins. This brings about a value for $E_L(L)$ which is higher than that of thiolate alone, concerning both interactions with phytochelatin and effective data for entire tissues. We are going to reconsider the dynamic (biocatalytic) implications of this later (Section 2.2.5).

2.2.4.2 (Lack of) Correlation and Differences in Biochemistry

As noted before (Section 2.2), the abundances of the elements essential for plants do not always correlate positively, which means there are some deviations from any possible general pattern of identical concentration relationships at either higher or lower levels.

Apart from necessities of efficient coupling of different metal-catalyzed biochemical pathways, e.g. in photosynthesis, there is highly positive correlation among most of these elements yet, holding for abundances of metals and non-metals in various plants alike (also cp. Fig. 1.3). Apart from this, biochemically relevant ligands will control resorption, retention and transport of metals according to Eqs. 2.1 0, 2.11 (below, section 2.2.5) all the way from rhizosphere upward to leaf tip or fruit (Figs. 2.6–2.8), implying that complex formation constants must be calculated using the electrochemical ligand parameters. In addition, metal fractionation is described by an effective electrochemical ligand parameter (Eq. 2.2) derived from the fact that entire plant organs tend to behave as if containing of a single, homogeneous kind of ligand. The latter values from sets of identical BCF values and Eqs. 2.4 and 2.10 are given in Table 2.7 below:

Table 2.7 Data for effective electrochemical ligand parameters of several different plant species. Values were calculated using Eq. (2.4)

Plant species	Tested organ	Groups of metal ions with equal $BCF_{soil/plant}$ values	Effective electrochemical ligand parameter for the metal ion group	Average value	Biochemical remarks
Lolium perenne	"Green" (above ground)	1. Eu, Gd, Tb, Er 2. Y, La, Nd 3. Ce, Dy, Yb		–0.19	
Taraxacum officinale	Leaves	Cd ≈ Cu; Sr ≈ Zn and Sr ≈ Cu ≈ Zn (other sampling site)	1. –0.29 2. –0.26 3. –0.27	–0.27	
Vaccinium vitis-idaea	Leaves			–0.04	The two *Vaccinium* species strongly differ in $E_L(L)_{eff}$ and usually do not coexist (competitive exclusion principle)
Vaccinium myrtillus	Leaves			–0.25	
Deschampsia flexuosa	"Green" (above ground)			–0.165	
Molinia caelurea				–0.135	
Betula pendula	Leaves	(a) [Grasmoor] (b) [grown from seedlings on sewage sludge in Lithuania]: Mn, Cu, Ni, Pb (BCF ca. 0.45 each) (c) upper Lusatia, including soil samples		(a) –0.17 V (b) –0.19 V (c) –0.20 V	Data from several, also polluted, sites (Lithuania) agree
Picea abies	Needles (1 year old)			–0.18	Mg/Mn ratio different from that of deciduous plants
Picea abies	Needles (2 years old)			–0.24	
Pinus sylvestris	Needles	1. Y, La, Pr, Nd, Sm 2. Gd, Tb, Er, Lu	1. –0.164 2. –0.13	–0.15	Concerning data for other parts of pine tree, see Table 2.15
Quercus ruber	Leaves	Many ions		–0.28	
Lemna trisulca	Total	La, Ce, Pr		–0.17	
Azolla filiculoides	Parts exposed to light	La, Sm, Eu		–0.23	Assumption: Eu present in divalent Eu^{2+} state
Ceratophyllum demersum		Ce, Sm		–0.17	

Data for birch and spruce trees, dandelion (*Taraxacum officinale* L.) directly correspond to soil analyses whereas the Ln^{3+} patterns of the data set provided by Markert are related to average REE contents of Central European soils. If Mg/Mn is about 5 already in soil – which frequently is the case – $E_L(L)_{eff} = -0.07$ V keeps this relationship also in the plant (photosynthetic organ); plants can produce this situation by delivering simple amino acids to the soil (rather than hydroxicarboxylates (citrate, malate)).

2.2.5 Translating Complex Stabilities into Bioconcentration Factor (BCF) Data: The k′ Term of Element Fractionation

It must be settled whether this approach is sensitive or insensitive towards nonphysiological distributions and amounts of heavy metals, not the least for the sake of biomonitoring. There are data on heavy metal (Zn, Cd, Cu, Pb) accumulation in litter and different soil layers and in plants of some oak woodland next to a metal smelter (Avonmouth near River Severn) in Great Britain (Martin and Bullock 1994). The concentrations in the litter layer are (in µg/kg): Cd 60, Cu about 170, Pb and Zn around 3,000. As usual for these metals except of Zn, where BCF ≈ 1 is a normal value, the concentrations of the four metals in photosynthetic organs of oak *Quercus robur*, other trees and scrubs and the fern *Dryopteris* are considerably lower than in supporting soils. In this restricted set of metal data, there are pairs of identical soil-leaf BCF only for *Quercus robur* (Zn, Cd; BCF = 0.045) and *Dryopteris* spp. (Cd, Cu; BCF about 0.13); the former corresponds to $E_L(L) = -0.28$ V, the latter to very similar –0.29 V.

Implication for biomonitoring: corrections by use of electrochemical ligand parameters and BCF-defined element clusters

Since the original data show that, before a prediction of BCF values of yet other elements in a given soil-dwelling organism can be made, the relationship between

- Empirical (sets of identical) element BCF values and
- The corresponding calculated (once again, identical) complex formation constants for bidentate binding

must be derived. The various BCF clusters for one species differ with respect to both BCF and to $-\log k_{diss}$ calculated from this for each set of equal-BCF-metal ions, with the quotient

$$k' = \Delta \log k / \Delta \log BCF \qquad (2.11)$$

that links both will depend on the corresponding species even if $E_L(L)_{eff}$ is similar in a couple of plant species, e.g., about –0.17 V. The size of this quotient k′ in addition gives some measure whether the plant might be able to hyperaccumulate certain metals which form particularly stable complexes with their biomass (-es): with $E_L(L)_{eff}$ being negative in general and $k' \approx \pm 0$, no hyperaccumulation will take place even concerning metals with either "exceptionally" positive (U, Cr(III), V(IV), Cu, Al) or negative (Nd, Ti, Zr) x values. Conversely, different values of $k' << 0$ or $k' >> 0$ may result in hyperaccumulation which, however, can only be realized if soil chemistry is appropriate. The value of k′ in addition gives a piece of information on the consecutive steps of metal (ion) sequestration and transport in some plant.

A cascade of ligands (sequestrants, protein carriers) with steadily increasing or decreasing $E_L(L)$ (possibly plus active transport through membranes) will produce corresponding bioinorganic amplication, notable by $|k'| >> 0$, which renders (hyper-)accumulation even of metals forming weak complexes possible (see below). Other series or sequestrants and carriers which do not produce a steady change of $E_L(L)$ from rhizosphere up to leaf tip or fruit, but "arbitrarily" change differences of metal ion attraction from step to step of transport, will exclude amplification, thus producing $k' \approx 0$.

Highly negative values of k′ in addition imply the possibility to retain or even enrich elements which will form but rather labile complexes given the effective electrochemical ligand parameter of the plant species and (Eq. 2.11). These include Sr, Ba or Mn and the REEs (except of Sm, Tb) if $E_L(L)_{eff}$ is close to zero in the latter case, all of which are known to be hyperaccumulated in some plants, e.g. Ba and Mn in Brazil nuts (Emsley 2001) and – among our test set of plant species – Mn gets substantially enriched in blueberries (both leaves (to which data reported here (Markert 1996) pertain) and fruits). k′ thus is a kind of measure for amplification of differences in the sequence of transport within some plant, from sequestration in/by root exudates to deposition in the tips of leaves,

Table 2.8 Sensitivity of BCF towards changes in complex formation constant according to Eqs. 2.1 and 2.3: $k' = \Delta \log k / \Delta \log BCF$. k' (positive or negative deviation from zero) denotes the propensity for hyperaccumulation of some element. As usual, data on REEs were used for scaling

Species	BCF for element cluster 1	$-\log k_{diss}$ for element cluster 1	BCF for element cluster 2	$-\log k_{diss}$ for element cluster 2	$\Delta \log k$	$\Delta \log BCF$	k'
Lolium perenne	0.016	6.82	0.007	6.30	−0.52	−0.36	+1.4
Betula pendula	0.004	6.82	0.011	5.99	−0.83	+0.44	−1.9
Vaccinium myrtillus	0.004	6.54	0.008	9.59	3.05	+0.30	+10.1
Vaccinium vitis-idaea	0.0045	6.82	0.007	8.36	1.54	+0.19	+8.1
Pinus sylvestris	0.004	6.10	0.008	6.64	0.54	+0.30	+1.8
Molinia caelurea	0.008	6.35	0.013	6.00	−0.35	+0.21	−1.7
Deschampsia flexuosa	0.0055	6.82	0.0105	5.97	−0.85	+0.28	−3.0

needles[3] or in fruits, respectively. In addition, a negative k' enables the plant to cope with high environmental levels of metal ion toxicants which form rather stable complexes, in particular, Al and Cu, whereas Ni and Zn are accumulated in specially adapted (serpentinite) flora (Lombini et al. 1998, also see Figs. 2.19a–c) rather than efficiently repelled. The values are given in Table 2.8:

In both kinds of *Vaccinium* there is potential for thorough amplification of small differences in complex stabilities within the plant and thus for hyperaccumulation; this mainly refers to *Vaccinium myrtillus* as its effective electrochemical ligand parameter is fairly low (−0.25 V). Grasses and trees (both deciduous ones and coniferes) vary around $k' \pm 0$, except for *Deschampsia flexuosa*.

2.2.5.1 Reasons of Biochemical/Biocatalytic Effects (Essentiality)

As pointed out before, one must strictly distinguish between distribution or accumulation of chemical elements in biomasses and their functions for the corresponding organism: the action need not be where the highest concentrations are but elsewhere, even disregarding inactive top concentrations in biogenic mineral phases such as bones or silica fibres fortifying plant shoots. Distribution and accumulation are related to the stability and variety of metal-biomass complexes (e.g. Wünschmann et al. 2004) whilst "function"

1. Can attain various reasons, e.g. catalytic, osmotic or – in animals rather than plants – related to communication among cells and
2. Requires some lability of binding – rather than inert coordination – in all of these functions

(except for some cases where the product is actively removed from the catalytic site by some secondary reaction or use of ATP – like in cleavage of NH_3 from nitrogenase Fe sites after reduction of N_2, CN^- or nitriles (Eady 2003) – an efficient catalytic cycle requires swift cleavage of the product from the catalytic center, likewise signal carriers (metal ions, organic ions like choline or amino acids binding to metal sites themselves) must not stick irreversibly to the receptor site which converts the signal, while osmotic activity takes both solubility and dissociation).

2.2.6 Binding Stability of Substrates and Products in Catalytic Cycles: How Does Ligand Sensitivity Influence Reaction Kinetics?

The end of this approach is to obtain such kinds of numerical values which can be used in QSAR approaches for describing both ecotoxicological (Schüürmann et al. 1994) features and aspects of elemental composition of biomasses in different species (Markert 1996; Kabata-Pendias 2002) and their various organs. This approach can be extended to entire trophic chains or networks, providing information on metal distribution throughout a biocoenosis, taking Eq. 2.4 as the starting point once again.

[3]Note that there is a difference in effective $E_L(L)$ of different ages in spruce needles.

2.2.6.1 Some Chemical Rules for Enzymatic Reactions

An efficient catalytic cycle takes

1. The ability of the catalyst (enzyme) to efficiently bind the substrate, that is, rather strongly and with fast kinetics, while
2. The product is easily cleaved or driven away by another substrate molecule/ion

This is a qualitative statement within a comparison; therefore some rules have to be defined whether any possibly catalytic reaction fulfils this criterion or complexation of educt(s) or/and product(s) do(es) simply interfere with the stoichiometric reaction or some step thereof, also influencing the turnover kinetics. This means analyzing – measuring, calculating, predicting – the interaction of some metal ion within a metalloprotein on one hand and substrates, products on the other. Ostwald's definition of a catalyst implies the metal ion and with it its coordinative environment not be changed permanently by this transformation which in turn requires the metal binding properties of the substrate and product to differ from each other.

In addition, some elements are essential for (almost) all organisms but may have different functions in them (e.g., Mo). Biochemical processes – including all those catalyzed by metal ions – take place within some polymeric matrix (the proteom) which mainly consists of chiral monomer components. Augmented by sterical effects, the reactions taking place in such media are very highly enantioselective. As a result, for catalyzing identical transformations of different enantiomers or diastereomers of the same substrate (e.g. D- and L-lactate or the set of hexose sugars) different, multiple enzymes are required (e.g., for metabolism of glucose in animals and man insulin is required but not for that of its enantiomer mannose or of the diastereomeric fructose). D-amino acids (other than methionine) in cell walls form an obstacle towards attack by bacteria. Therefore, though a "counting" of similar functions due to one biocatalyst is required for SNA (chapters 2.2.8, 2.2.9), one tends to underestimate the multiplicity of unsubstitutable functions.

The catalytic reaction means the system to become **dynamic**: a neutral or anionic substrate directly binds to the metal center of an enzyme (which can be hard or soft itself), to undergo some chemical reaction there which likely also changes its polarizability. A "hard" ligand will bind to a hard center such as Mg^{2+} to become an, at first stably bound, substrate of some catalytic reaction; if the product is "softer" than the educt, it is likely to be cleaved from coordination after this reaction. Haloperoxidases give an example how this mechanism operates: hydrogen peroxide, being a "hard" ligand like water, gets bound to hard centers like V(V) or Fe(III), the latter forming a porphyrine complex (haem). The Fe porphyrin will thereafter react with "soft" halide ions (Rehder 1991; Frausto Da Silva and Williams 2001) which do (need) not at all bind directly to the hard metal center, thus avoiding formation of a hard–soft pair during this transformation. Nevertheless the hypohalogenite intermediate, the arene/PAH cosubstrate and the phenolate/haloarene product (it is unlikely that the arene/PAH ever comes within binding range to the V or Fe center during this oxidation, and the same holds for the haloarene which in addition is a very poor ligand) are all softer than both water and hydrogen peroxide, allowing for substitution – and accordingly for closing the catalytic cycle – by another oxidant attack or simple hydrolysis. Theoretically speaking, hypohalogenites such as OCl^- or OBr^- might behave as ambidentate ligands here, much like CN^- (Balahura and Lewis 1976) or more oxygen-rich non-metal anions such as SO_3^{2-} (Burmeister 1968) or AsO_3^{3-} (Fränzle 1991, unpublished), but binding via halogen was never observed with $OHal^-$ or more than just postulated with ClO_2^-.

Now consider a soft metal center (monovalent Co, Ni or Cu) which promotes transformation of some soft ligand substrate to yield a "harder" product; here likewise another substrate molecule or ion will remove the product from the ligand sphere. An example of this is given by both Ni-dependent and "Fe-only" hydrogenases; the structures of their catalytic centers and of some biomimetic model compound are depicted in Figs. 2.3 and 2.4:

By itself, the dihydrogen molecule is soft and very well polarizable. The extent of softness in **hydride** is shown by an unique feature: even by but slight cation-mediated polarization, its radius of some 208 pm may shrink by 30–40% (Cotton and Wilkinson 1981). After getting into a hydrogenase enzyme molecule, a strongly basic arginine (that is, guanidine) residue cleaves a proton, leaving behind an extremely soft hydride ion H^- which gets bound to a – likewise soft – Ni(I) center. When H^- is oxidized to yield OH^- or water thereafter, it still acts as a ligand but the latter now has become

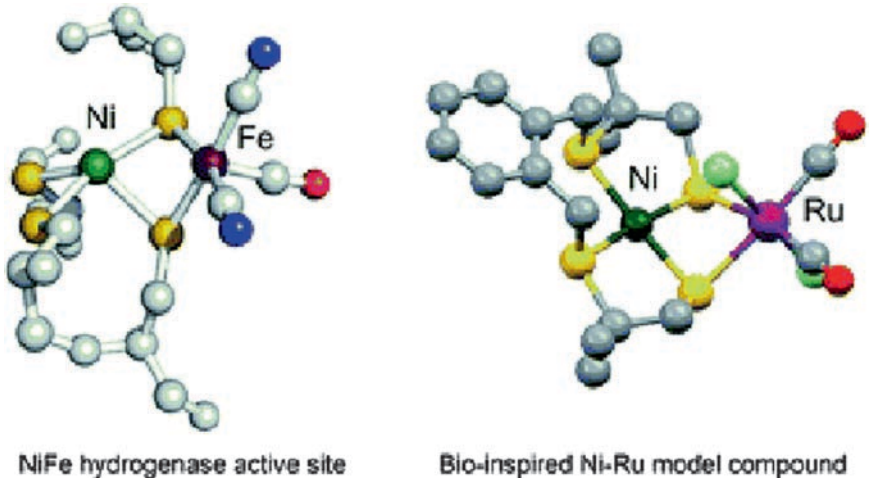


Fig. 2.3 Simplified, schematic representations of the active centers of NiFe-hydrogenases (left picture) and of some model compound using Ru/Ni as an H acceptor (to the right). In NiFe-hydrogenases, Fe is coordinated to two cyanoligands and one CO (carbonyl), in addition there are two thiolatobridges. Ni, on the other hand, is linked to sulfur (cysteine residues, i.e. thiolate) only except for one oxo- or – if protonated – hydroxobridge which is "hard" and accordingly replaced by hydride after heterolysis of the H–H-bond in H_2. In "Fe-only"-hydrogenases both Fe are bound to CO, but in an asymmetric fashion: one in addition bears cyanide whereas the other is linked to thiolate once again. Owing to this difference in complexing ligand environments, the redox potentials of the two Fe ions differ considerably (by some 0.4 volts, for Ru(II/III) the difference should be 0.97 V). Both the CO ligand and thiolate(s) are capable to form (very soft) bridges towards another metal ion (Fe) which also is tethered to the protein matrix by means of complexation of amino acid side-chains (i.e., not like members of some Fe_xS_4 cluster). The enzyme operates by heterolytic scission of the H–H-bond of dihydrogen (cp. Fig. 2.4) to yield a proton, thereafter accepted by the protein matrix (e.g. by a lysine or arginine moiety) and a hydride ion. Hydridoligands thus formed bridge 2 Fe (much like they do in boranes, e.g. B_4H_{10}) or are trapped by Ni and can then be transferred to hydride acceptors like pyridinium salts (NAD^+) or be oxidized directly, in situ

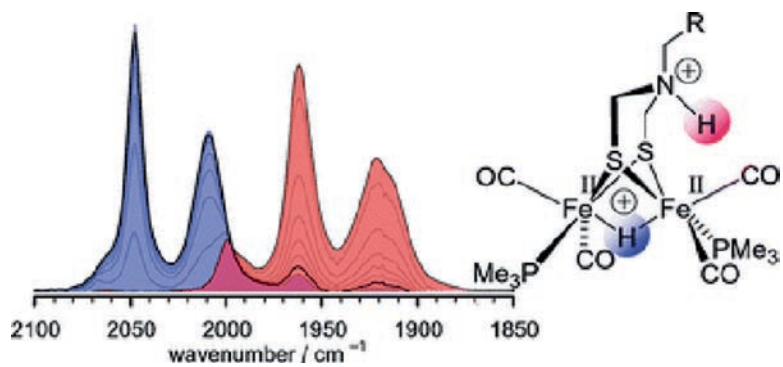


Fig. 2.4 Hydrogenase model by Schwartz (2009). As compared to the "natural" cyanide, trimethyl phosphine ligands in the "model" complex will increase redox potentials of the Fe(II) centers while reducing M-H acidity of hydride (cp. Fränzle 1992). This diminishes the tendency of both hydrogen atoms to get oxidized (thus stabilizing the state of this model vs that of native hydrogenated 2 Fe-hydrogenase). H_2 heterolysis is also augmented by some basic neighbour group (the secondary amine), like in biochemistry

hard and accordingly is far more weakly bound to either Ni(I) or Ni(II) than any of the other surrounding ligands, i.e. thiolate (cystein residues), CO or cyanide. The former two in turn can rearrange into ligand bridges, thereby cleaving the "hard" product water. Ni(I) may appear as an uncommon oxidation state but is jointly stabilized by CO and CN^-, and thus Schubert described model complexes (not yet dubbed so in those times) combining CO, cyano and thiolato ligands at Co or Ni as early as 1933 (cp. Albracht et al. 1986). Thiolato bridges which can open and close again between two metal ions are very soft, accomodating

hydride, etc. but readily extruding aqua ligands if the metals are soft. Recently, a first functional model of a "Fe-only" hydrogenase was described by Schwartz (2009); here, an amine removes the proton with hydride once again interconnecting (bridging) both Fe ions. Some pieces of information on structure and vibrational spectra of this complex are given in Fig. 2.4:

The product may also be cleaved readily if it is a stronger (Brønsted) base than the substrate, if polarizability is poorer or ligand properties are almost lost because there are no more free electron pairs (e.g. with CO-oxidase producing CO_2). With the product being "softer" than the substrate, being bond less tightly to a hard metal ion such as Mg^{2+}, a catalytic transformation is feasible there, with some soft ion like Cu^{2+} doing the same for substrates which become "harder" during the reaction. Although this approach provided logical, comprehensible results for H_2 or hydride, respectively, it is restricted to quite a couple of very small molecules or ions undergoing biochemical transformations:

There are substantial differences between polarizabilities of substrates and products only if the molecules or ions are very small (substrates H_2, CO, CO_2, N_2, CN^-, R-NC, NO_2^- and products water [hydrogenase], CO_2 [CO oxidase], HCO_3^- [carboanhydrase], NH_3 or CH_3NHR (R = alkyl, aryl; nitrogenase), or NO, respectively). Here, and only here, Pearson's qualitative argument provides clues whether rather hard or better soft ions may catalyse some reaction, but there still is no information on which particular metal ion in a catalytic center would be best-suited. For larger or even macromolecules which so often undergo reactions in biochemical systems, even that qualitative statement becomes impossible. The distinction of "hard" and "soft" classes thus just allows for identification of some larger sub-group of potentially suitable (bioinorganic) catalysts. There is no real contribution to understanding reasons of essentiality of certain elements or their ions; thus one has to abandon the HSAB approach for this purpose even though it yielded meaningful explanations with some cases such as hydrogenase. It will be replaced by an empirical two-parameter-description of metal ion properties given in Eqs. 2.4 and 2.6 and its mapping to essentiality, also allowing for quantitative calculations on both the substrate and product binding to a given ion. This can be achieved without formulating some general concept of how such metal ion properties influence substrate activation and contribute to essentiality (Figs. 3.1 and 3.2), which would be very difficult to do in a general way.

2.2.6.2 Enzymes Acting as Catalysts

Mostly biological processes and the chemical transformations involved there do not occur on a mineral support but rather at catalytic centers (some 30–40% of them containing metal ions (Höhne 1980; Williams 1983; Williams and Frausto Da Silva 1996)) embedded into some organic matrix. Comparing the list of essential elements (Table 2.1) to the multitude of possible inorganic (or organometal) catalysts known to be feasible and efficient for promoting those transformations relevant for biochemistry (and used or successfully tried in technical catalytic chemistry), there are obviously restrictions cutting back their variety and number. Why this difference? There are (at least) two principal distinctions between biological and technical organic chemistry even though both employ catalysts for same or very similar reactions:

- In bioinorganic chemistry, reaction conditions are restricted: aqueous or membrane-confined medium; 0.2 bar < p < 1,150 bar; 265 K < T < 386 K (possibly 394 (Kashefi and Lovley 2003)) (<355 K except for cyanobacteria and archaeae). This maximum pressure essentially corresponds to the pressure at the ultimate depths of the (Pacific) ocean in the Mariana trench. Down there, metazoa (holothurians, small crustaceens) still survive (fishes (Abyssobrotula) exist only down to some 8,400 m) but it is known from experiments that the pressure limit for reproduction of bacteria is about the same (the hyperpiezophile *Shewanella benthica* grows up to 1,048 bar (Wharton 2002)), allowing for sterilization of samples – and food conservation – by simply applying even higher pressures.
- Second, there must be reproduction, corresponding to autocatalysis (see above): there is life only (*by definition*) when there are reproducible structures and entities, regularly making "copies" of all organs, cells and other sub-units.

For a "normal" chemical system, there is autocatalysis if the reaction is catalyzed and affords additional catalyst as a by-product. Biological systems behave autocatalytic by cell budding and reproduction of the entire organisms (if metazoic). This implies to reproduce

all the subordinate functional units – down to single molecules.

Thus, autocatalysis occurs in biology across various levels of structural and functional hierarchies, from a complete plant (or fungus, animal) over single cells down to such molecules which take part in metabolism and get doubled in cell budding and reproduction also. Both the

- Molecules (daltonic species of "formula" $C_xN_yP_zH_aM_bS_c$ …) and the
- Entire organisms are distinguished by well-defined, constant chemical compositions (the basic statement of ecological stoichiometry (Sterner and Elser 2002))

Accordingly reproduction of some organisms is tantamount to increasing the matter turnaround without changing the composition of the autocatalytic system, except for proteins slightly altering their sugar "covers" (isoenzyme species, immunological responses), otherwise it would no longer be **auto**catalysis. The use of chemical elements as biocatalysts represents autocatalytic reproduction of the catalysts, e.g. containing some metal; thus this level of hierarchy can be selected for theoretical analysis. Almost all transformations in a living being are catalyzed by some enzyme or co-factor but not all of them are anabolic, producing additional catalytically active biomass but might simply afford energy (thereby changing C/N/P/metal ratios of the inflow (food) to those of the respective organism (+ egesta) by emitting CO_2, urea, etc.). Therefore it is tempting to analyze catalytic chemistry of living beings by using some theoretical approach which by its very structure distinguishes between "simply catalytic" and autocatalytic processes, in addition analyzing whether the latter ones can be sustained. Such an approach suited to distinguish between "colloquial" and autocatalysis is **stoichiometric network analysis** (SNA (Clarke 1975, 1980), see Section 2.2.7).

Generally, for an efficient catalytic cycle certain conditions must be met: the catalyst has to

1. Get into some chemical interaction with the substrate (compound or mixture to be processed), either binding it covalently (e.g. by oxidative addition) or at least adsorb it to the interface transferring some charge, then
2. Enhance reaction rates by reducing the activation energy barrier, e.g. by altering cumulations of charge (bind polarities) and thus favouring reactions which entail or require charge exchange (redox processes, reactions with ions, e.g. hydrolysis). Eventually
3. The chemical or chemisorptive bond between product and catalyst must be readily cleaved after having effected the transformation (the opposite case being called either "catalyst poisoning" (technical) or "product inhibition" (biochemical)).

Thus, the product must not bind to the catalyst (the enzyme) as strongly as the substrate or additional reaction partners (oxygen, water). In many cases of enzymatic or carrier reactions which mediate stoichiometric transformations or transport some species by reversible chemical reactions (dioxygen, methyl groups, etc.), metal ions act as necessary reagents in the center of the catalytical/chemical system. We do not consider regulation of enzyme activities here caused by metal ions such as Ca^{2+} which bind to the "outer side" of some protein and change conformation/tertiary structure of the protein in a way as to open or block access to the reactive pocket (e.g. Williams 1983). By means of association or complex formation constants, interactions among such metal centers and the substrates or species to be transported can be described quantitatively (McLendon and Martell 1976 for competitive O_2, CO or NO binding; Williams and Frausto Da Silva 1996) which also holds for the products, thus testing criteria 1 and 3 given above.

2.2.6.3 General Features of Metabolic Kinetics

In order to investigate the implications of Sabatier's principle for drawbacks of different complex stabilities in a minimum model enzyme (some metal ion promoting a biorelevant transformation without any protein matrix involved or another doing catalytic chemistry with the central ion replaced by others), two model reactions from the (older) literature are considered, namely metal-induced hydrolysis of ethyl glycinate (an amino acid ester) and activities/turnovers of reconstituted carboanhydrases. For the esterase activity at ethyl glycinate, the reaction rates apparently depend on ligand sensitivity x_{1d}, while the well-known high efficiency of Zn^{2+} as promotor ion is related to its relative properties in the second case, showing the range of conclusions available by this simple approach.

Some "classical" metalloproteins such as the above Zn-dependent carboanhydrases and carboxypeptidases

Table 2.9 Rates of hydrolysis of ethyl glycinate at pH 7.9 (for Cu(II): pH 7.3) promoted by different divalent ions (Kroll 1952) depending on c- and x-parameters. To compensate for a smaller contration of hydroxide, hydrolytic rates at/with Cu(II) must be multiplied by four (values in brackets)

M^{2+}	k	k_{rel}	log k_{rel}	c_{1d}	x_{1d}	c_{2d}	x_{2d}
Ca	0.0007	1	0	–	–	0.73	−10.98
Mg	0.0007	1	0	−0.1	−4.6	3.94	8.24
Mn	0.00351	5.0	0.70	0.3	−5.2	3.01	−5.32
Co	0.0156	22.3	1.34	1.17	−5.3	5.48	3.93
Cu	0.0425 (0.170)	61 (244)	1.78 (2.37)	1.70	−8.0	9.04	21.84

are particularly well-studied, and data for equilibrium constants of competitive inhibitor binding, those for the original substrates and the products are also known (Lipscomb 1982; Kimura 1979). Like with the aquaion, relative affinities of Zn^{2+} within this biopolymer towards various monodentate monoanions like azide [$E_L(L)$ = −0.30 V], thiocyanate [$E_L(L)$ = −0.06 V if N-bound (M-NCS)] or acetate [$E_L(L)$ about −0.22 V] show that c and x for binding of such simple ligands resemble that of the aquaion, x being negative, too. In the above metalloproteins, Zn^{2+} is surrounded and coordinated by three imidazole moieties (histidine residues) while x_{1d} = −8.93 for the aquaion (Table 2.3).

Now take some closer look into carboanhydrase (CA) mode of operation, carboanhydrase being of principal importance in photosynthesis especially of aquatic plants: HCO_3^- coordinates to Zn-CA with log k_{ass} = −log k_{diss} = 1.6 (Kimura 2001). c and x values (monodentate) of the proton in anionic acids and the acidity constant of HCO_3^- (pKa = 10.46) are combined to obtain $E_L(L)$ for HCO_3^-, namely, −0.37 V which provides for the observed bidentate association of HCO_3^- to Zn^{2+} −log k_{diss} = 1.93, closely matching the above value even though it was derived for the simple aquaion. If Zn is replaced by Co^{2+} or Cd (Price and Morel 1990; Strasdeit 2001) in CAs, −log k_{diss} increases to 4.03 (Co) or 2.2 (Cd), meaning that bicarbonate from CO_2 hydration will be more strongly retained by the enzyme than with Zn. It is conspicuous that "alternative" Co- or Cd-dependent CAs operate in seawater (i.e., marine microalgae) with its considerable carbonate content (≈2 mmol/L; Nozaki 1997) and alkalinity (pH = 8.3 (Sillen 1967)), facilitating the reverse reaction and forthcoming CO_2 transfer to ribulose-bisphosphate if there is an effective source of protons. The stability of the Co intermediate would then require a HCO_3^- concentration of some 10^{-4} M at pH 8.3, that is, total carbonate ≈0.015 M or 15 mmol/L, somewhat more than in the ocean. Accordingly, cleavage of product from enzyme still is feasible using Co-exchanged CA in seawater, fully meeting observations (in vitro: Vallee and Williams 1968; in vivo: Price and Morel 1990).

The other, older model system is the metal-induced hydrolysis of glycine esters (Kroll 1952); it must be pointed out that, due to the stability of the forming aminocarboxylate complexes, the reaction is stoichiometric rather than catalytic with respect to the metal ions (almost irreversible formation of $[M(glyc)]^+$). As shown in Table 2.9 above, turnover rates correspond to/increase along the Irving–Williams series, but there is no straightforward relationship with either c_{2d} or x_{2d} values, Mg^{2+} being as inactive as Ca^{2+}, but rates increase with falling c_{1d} and x_{1d}. Thus the process probably starts by amine-like monodentate coordination of ethyl glycinate, with some water ligand cleaving the O-C_2H_5 bond while the M-O(H_2) coordination remains intact. Therefore, there is no relationship of hydrolytic kinetics to self-exchange rates of water (Merbach 1982; Jordan 1994) at the corresponding metal ion center.

Also in other transition metal ion systems, glycinate ligands tend to close the chelate ring rather slowly, e.g. with Ni^{2+} (Jordan 1994). A correlation with x_{1d} yields

$$\text{Log } k_{rel} = -0.615 \times x_{1d} - 2.448 \qquad (2.12)$$

which can be linked to the Arrhenius approach for activation energies

$$\text{Log } k_{rel} = -2.303\, E_{act}/RT + \log a \qquad (2.13)$$

with a being the abundance of potentially reactive "hits" among the reaction partners (here: ethyl glycinate and water/hydroxide polarized and activated by complexation to M^{2+}), accordingly

$$-0.615\, x_{1d} - 2.448 = -2.303\, E_{act}/RT + \log a \qquad (2.14)$$

which can be rearranged to provide

$$E_{act}/RT = 0.267\, x_{1d} = + (\log a/2.303) + 1.063 \qquad (2.15)$$

for this reaction. As x_{1d} is negative in all the above cases and a should be close to constant, the first term

0.267 x_{1d} gives the reduction of activation energy directly (although the absolute value remains unknown) while (log a/2.303) + 1.063 just is a constant. At T = 25.4°C, E_{act} will reduce by (−3.4) × 0.267 × RT ≈ 5.2 kJ/mol between the catalytically inactive Mg and Ca ions and Cu^{2+} if a and thus log a is constant. Any dependences of a from, e.g. different self-exchange rates at the different metal ions are "hidden" in the data.

By Mn(II) and Mg, Kroll used two of the more frequently occurring metal ions in hydrolases, but omitted Zn^{2+} which actually prevails (which was not yet settled in 1952). So, if a straightforward extrapolation to real peptidases or esterases is feasible, the yet lower x_{1d} of Zn^{2+} of −8.93 will provide an advantage; however, at −0.29 c_{1d} also is much smaller than with Cu. Correspondingly for the amine-type intermediate $-\log k_{diss} = -1.0$ which is to say that the amino acid ester is reluctant to bind to Zn^{2+} at all (Cd would be rather better suited; $-\log k_{diss} = 0.5$). Thus ester hydrolysis by metal ions having very negative x_{1d} values like Bi^{3+} or the REE ions will not work for amino acid esters even though they might be (and sometimes are indeed) pretty effective in phosphate cleavage.

Let us finally consider implications of these findings for reaction mechanisms in metalloproteins. Therefore, we must take into account that, much like with Sabatier's approach, considerations about thermodynamic stability, which might go as a static phenomenon if it were not for the fact that chemical equilibrium is nothing but the ratio of "forward" and "reverse" reaction rates, hence it also is about dynamics and might be compared to other reaction rates, this approach being encouraged by the well-known structure–reaction rate relationships for both (at least benzenoid aromatic) substrates and square-planar or octahedral coordination complexes:

(a) The Hammett rule of kinetics of (electrophilic (Hammett 1973) or radical-based (Kwok and Atkinson 1995)) aromatic substitutions states that the logarithm of reaction rates in 4-substituted benzenoid aromatics directly depends on some substituent constant σ_p provided the kind of reaction, solvent and temperature are kept constant (e.g. hydrolysis of benzoate esters in 50/50 aqueous acetone at 50°C or bromination of phenols at RT in neat $CHCl_3$), formally: $\log(ki/k0) = \rho\sigma_p$ (Hammett 1973)

(b) Substitution at, e.g. Pt(II) centers likewise can be described using a formally identical equation and empirical parameters describing the attaching group (standard conditions: methanol, 30°C (Tobe 1976; Jordan 1994)); the values of this parameter (which is 0 *by definition* for methanol itself, positive for all the others, e.g. SCN^-) mainly depend on identity of the incoming donor site atom, less on its local charge.

(c) The values of the electrochemical ligand parameter also follow Hammett's rule (Fielder et al. 1995) with regard to both first-order and second-order terms (first-order refers to direct binding of Hammett-type "substituent" Su^- with one additional electron to some metal ion like Ru(III) – Ru-Su^- as a ligand rather than via aryl side chains, e.g. as Ru-N(*para*-$C_6H_4Su)R_2$ – whereas second-order means the opposite case concerning, e.g. meta- and para-substituents of monodentate ligands, for example, M-P-binding aromatic phosphines Ph_2P[4-R-aryl] or P(4-R-aryl)$_3$ or 3- and 4-substituted [4-R = t-butyl, Me_3Si up to CN, -NO_2, NMe_3^+ and –COOR′] pyridines and 4-alkyl pyrazinium ions acting like pyridine ligands). In either case the ligands cause potential shifts which are linearly correlated with the now classical σ_p values of Brown and Okamoto (1958) (Fränzle, unpublished work using Lever's table of $E_L(L)$), and by Eq. 2.4 the same holds for complex formation equilibria.

(d) Change of ligand charge by protonation/alkylation or deprotonation at the α-position of donor sites, e.g. in the cyano/HNC- or cyanamide/M ← NC-NR^- or /M ← NC-NR_3^+ or dinitrogen/N_2R^+ ligand couples, changes $E_L(L)$ by about 0.4–0.6 V while effects at rings (salicylate anion (neutral phenol site)/dianion or hydroxamic acid/hydroxamate) are smaller (≈0.2–0.25 V). Assuming M-L bond lengths of some 200 pm (2 Å) and local dielectric constants in the coordination sphere of ≈15 like in semiionic materials (SiO_2, GaP, many other semiconductors), the merely electrostatic contribution to $E_L(L)$ change should be Δε = 0.48 V caused by introducing or removing electrostatic attraction between M^{2+} and L^-.

(e) Turnover or exchange of ligands at square-planar or octahedral metal ion centers depends on the so-called *trans*-effect (Tobe 1976) which latter is a kinetic phenomenon once again: exchange rates of a ligand in the *trans*-position are enhanced by binding of some "trans-active" ligand bound to the very metal, and the extent of this effect also depends on the metal center: in, e.g. Al complexes

it is negligible while it is very pronounced with Pt(II) (square-planar) or Pt(IV) (octahedral). The *trans*-effect of some series of ligands steadily increases with electrochemical ligand parameter: $F^- < Cl^-$, $Br^- < I^- < CN^-$ (anions) or NH_3 < pyridine < RC**N** < phosphines < CO (neutral ligands), grossly increasing with an increasing $E_L(L)$ (between −0.42 (fluoride) and +0.02 V (cyanide) and +0.08 (ammine) up to 0.99 V (CO), respectively). This agrees with both the interpretation of the electrochemical ligand parameter in terms of perturbation theory advanced by this author and the interpretation of the *trans*-effect as to be due to M-L-^{trans}L′-π-bonding interactions given by Syrkin (1947) and Chatt et al. (1958) (see Coe and Glenwright 2000).

As a result of this and Eq. 2.4, Hammett constants directly influence the complex formation equilibria, although to varying extents depending on whether this is first-order or second-order, the latter due to aromatic substitution next to different donor sites (P (phosphines), N (benzamides, pyridines or pyrazinium ions), S (thiophenolates) or O (phenolates, benzoates, combinations like salicylate derivates) or combinations thereof (see case c. above) (salen derivatives or those of hippuric acid, both linked to metal by N + O)).

Accordingly, Hammett constants or combinations of the arguments a and b can be used to estimate both complex stabilities of substituted aromatics with donor site atoms other than C in biology/biochemistry. Therefore the connection between the "rest" of the protein and tyrosine phenolate, tryptophan indole or histidine imidazole moieties is taken to be one huge substituent (e.g. in the frequent cases where three histidine residues coordinate to one copper or zinc ion or with tyrosine residues in water photooxidation), possibly introducing charge effects by pH, phosphorylation ("P switch") or by complexation of other metals like Ca on the outer periphery of the molecule.

Then, knowing x, the stability of intermediates and product inhibition both depend on the substituents. So much for details of enzymatic reaction kinetics as depending on details of metalloprotein structure; similar second-order effects are observed in alkyl fluorophosphates hydrolysis by various Cu(II) complexes containing different chelating N-donors (Wagner-Jauregg et al. 1955); here the rates and turnover numbers increase with $E_L(L)$ of the N-chelators. In the above example of a simple model catalysis for amino acid ester hydrolysis – and, in fact, in the center of this discussion – we are rather concerned with effects of variation of the metal ion in the catalytic center itself, effects depending (or not) on differences in c and x parameters describing metal ion center-substrate interaction while here – like in reconstituted apoenzymes – the organic macroligand is kept constant or, like in this model case, is non-existent. From the above data it is obvious that reaction kinetics of hydrolysis correlate with x_{1d} but not with intrinsic bond strength c_{1d}. Arguably the dependence on x_{1d} represents the effect of change of charge and M-L bond network structure upon hydrolysis of the ester after its complexation. This does suggest that the metal ion does exert its effect rather early on the reaction coordinate, i.e. just after complexation of the substrate, rather than kinetics be controlled by retention of the product. Mg^{2+} – like Ca^{2+} – does not cause a catalytic turnover in amino acid ester hydrolysis as neither forms an ammine complex in aqueous solution while the Mg glycinate complex is rather stable.

As was mentioned above, the *trans*-effect depends on $E_L(L)$ and itself controls ligand replacement, its size (capability to alter exchange kinetics) being significant among "biometals" with Ni, Cu and possibly Fe, Cr centers. Thus, hydrogenases (with their peculiar CO and cyano ligands at Ni or/and Fe) and some other oxidoreductases (arene/phenol hydroxylases, amine oxidases, SODs, etc.; all containing Cu, mainly shrouded by imidazol (histidine residues)) should be reasonably sensitive to this *trans*-effect, and accordingly there is hardly any variation – except for introduction of one OH/O^- (serine, threonine, tyrosine residue) at Cu(II) bridges – of the ligand arrays around such catalytic centers. Hydrogenases, then, operate best in a ligand environment of very high *trans*-effect, while type-II or type-III "non-blue" Cu oxidoreductases (including N_2O reductases and superoxide dismutases) or hemocyanin are best at intermediate *trans*-effect, $E_L(L)$ for imidazol being but slightly higher than that of amines but considerably lower than for pyridines or nitriles.

This apparent optimum situation for oxidoreductases or O_2 transport (corroborated by model complex studies each) markedly differs from that for hydrolase/phosphatase activity (organophosphate cleavage; Wagner-Jauregg et al. 1955) or for autocatalytic peptide formation associated with Cu(II) (Le Son et al. 1998), the first akin to Sabatier- or volcano-curve

catalytic behaviors (Rothenberg 2008) as electron transfers are involved while maximum *trans*-effect is better when (non-redox) polarization of carbonyl or phosphate ester bonds matters.

As evidenced by the redox potential increase associated with complexes of the latter high-*trans*-effect-ligands, there is minimum electron shielding at Cu with these ligands, facilitating polarization and activation of ester bonds or amino acids, respectively while there is an optimum redox potential for catalysis of a certain transformation which need not be far higher than required for the given transition (and *reversible* O_2 binding will also occur if and only if the Cu(I/II) potential is neither too low nor too high), rendering some "intermediate" potential and thus *trans*-effect extent best for biocatalysis. Before specifically re-addressing the apparently different case of hydrogenases, let us cast these qualitative arguments and reasonings into the familiar formalisms of enzyme kinetics: the **Michaelis–Menten** description.

2.2.6.4 Michaelis–Menten Kinetics

Michaelis–Menten kinetics and empirical kinetic parameters can be joined to give pieces of information on optimum (diffusion-controlled) catalytic kinetics, just assuming that optimum turnover means use of the best catalytic center species and half substrate saturation. This holds for small substrate concentrations, probably subject to intracellular control or limited take-up. Then:

$$(1/V_i) = (1/V_{max}) + k_m/\{V_{max} \times [S]\} = (1/V_{max}) \times (1+ k_m/[S]) \quad (2.16)$$

or

$$V_i = v_{max} \times [1/\{1+k_m/[S]\}] \quad (2.17)$$

With k_m being the **dissociation** constant ($+\log k_{diss}$) of the substrate-enzyme complex; for a metalloprotein with direct coordination of substrate S to the catalytic center (or in cases where nucleophilic substrates are retained by non-metal electrophiles such as aldehydes (sugars), H-bond donors, boron compounds for which c and x parameters are known) this is identical with Eq. 2.4. Thus k_m can be represented using x and c values, and the largest possible turnover at a given substrate concentration implies k_m/S to approach zero, and hence, as [S] cannot be larger than a few M/L in aqueous media at most (usually much less), dissolved O_2 e.g. some 300 μmol/L, k_m and accordingly $+\log k_{diss} = \log k_m = -c - x \times E_L(L)$ must not become too large. $K_m/S \rightarrow 0$ means $\log (K_m/S) = \{\log k_m - \log [S]\} \rightarrow -\infty$, defining an **upper limit of an acceptable complex stability** for purposes of biocatalytic efficiency (cp. the toxicity of ions like Be^{2+}, Ag^+, Tb^{3+}, Pu^{4+} or UO_2^{2+} which, at "average" values of x_{2d}, have exceptionally high c values; Fig. 3.1).

One might add data for [S] or log [S] to the lists and mappings given in Table 1.1 and Fig. 3.1 depicting "optimal" agents of biocatalysis, however, except for archaea living in mine tailings and the like, [S] << 1 M/kg and thus $\log k_m$ should likewise approach ↑ , notwithstanding the necessity of prior complexation.

2.2.6.5 Steady States in Metal-Promoted Biochemistry

Living beings are throughflow reactors in which long chains or even cycles of reactions occur most of which are catalyzed by enzymes. As a rule, there is a constant level of element concentration involved in biocatalysis; for example, in humans it is about 1 attomole/function per cell, distinguishing a concentration/amount c_i for the given reaction i and a corresponding kinetics (if catalyzed in this manner) k_i. Accordingly, the optimal state of biocatalysis in some chain or cyclic reaction $i \rightarrow j \rightarrow k \rightarrow l \rightarrow m \ldots$ (or i again) is given by $k_i \times c_i \approx k_j \times c_j \approx c_k \times k_k \ldots$, with $k_{x(M)}$ being a complicated function of substrate, product and catalyzing metal ion center (see below, Section 2.2.9 and Eq. 2.33). Now the window of essentiality (fig. 3.1) provides a kind of borderline inside which the product $k_i \times c_i \approx k_j \times c_j \approx c_k \times k_k$ is sufficiently large to sustain metabolism (provided temperature, pH and administration of nutrients, trace elements and energy sources are adequate) but outside it will be (too) close to zero. As it is a product, either term of $k_i \times c_i$ may become too small. One sufficiently large k_i will not do due to the three-functions-rule but at least two other $k_x >> 0$ are required while c_x will only differ from c_i when complexation (the ligand sphere in terms of biocoordination chemistry) of the metal in various metalloproteins will differ. On one hand, there are metals which are (very) abundant (Al, Ti, etc.) but lack sufficiently many different catalytic

"options" whereas others are/provide most versatile catalysts but are much too rare (platinum group metals); the actual concentrations of PGMs in plant biomasses are even two orders of magnitude lower than was thought up to the early 1980s (Markert 1996). Yet other metals may be rather active catalysts but too selective (e.g. when REEs act as phosphatases) although they are not extremely rare, still placing them outside the window of essentiality. Finally there are some metals located inside but fulfilling biocatalytic functions never (Eu) or only in certain organisms (Cd, V, Ni, to different extents).

There are reasonable arguments for every metal ion with an appropriate c/x-combination being capable to catalyze quite a number of pertinent reactions, so – except for europium which is an ultratrace metal – limitations might arise from positions in trophic chains or metabolic features (requirement for vanadium to catalyze hydroxylating oxidations) only. Ni is and Cd may be used in plants even more than in animals or fungi. If there is mutation somewhere in the apoprotein sequence, electronic conditions and thus catalytic properties, particularly in hydrolytic processes, will modify kinetic features of catalysis c_x but also – to a smaller extent – k_x while very weak complexation (like with Ba or Sr or alkali metals) will make c_x essentially vanish also. This is the reason for the lower (leftward) limit of the window of essentiality (fig. 3.1) even though metal ions like Ba^{2+} are pretty efficient in transformations among sugars and polyaldehydes. Vanadium in animals probably is the most rare of the essential biocatalysts (plants contain considerably more of it but to no biocatalytic effect), its complexes not being too stable in either tri- or tetravalent states, and a similar situation holds for Co^{2+}. Thus Co and V can be taken to estimate how large c_x must be at a minimum, namely ≈0.1 μg/g dried biomass (≈2 μM/kg DM). Both V and Co are located next to the right-side edge of the window of essentiality and thus probably k_x will not be too large either while there is diffusion control in many other, especially Zn-based cases of metalloenzyme activity (Wolfenden and Snider 2001). It is impossible to infer this the other way round as there is no way of deducing catalytic properties from simple physicochemical properties of catalyst species. The minimal value of the product $k_i \times c_i \approx k_j \times c_j \approx c_k \times k_k$ for all ≥3 functions and thus k values must thus be of order 10^3 s^{-1} in green plants or animals. This product has the dimension s^{-1} (μg/g concentration times moles of substrate turnover/second per mole of enzyme).

The kinetic consideration outlined here is very similarly based on empirical properties of metal ions which (although loosely) correspond to their σ- and π-bonding capacities and – directions like with Hammett's approach (Hammett 1973; Schüürmann 1991) for reaction kinetics in benzenoid aromatics. In either case, there is no precise link as yet with electronic parameters closely defined in quantum chemistry like charge density, as the principal objective by now is to understand reactions kinetics and thus selection of certain catalysts, discriminating against others.

Now let us re-address the issue of hydrogenases and of the enzymes which catalyse a kind of backward reaction, namely the CO oxidases, investigating the release of H_2 from water at appropriate potentials (often these enzymes are called "CO dehydrogenases" but there is no hydrogen in CO that might be removed at all!). Both CO and H_2 display "normal" values of aqueous solubilities, i.e. [S] ≈ 10^{-4} M/(L × bar), thus should held $k_m \leq 10^{-5}$. E_L(L), likewise, is known for all the ligands involved, +0.99 V for CO, 0.04 V for water and some –0.30 V for (terminal, non–bridging) hydrido (H^-) ligands. As mentioned before, hydrogenases contain carbonyl and cyano ligands bound to Fe besides thiolate (Figs. 2.3 and 2.4), and there are several 3d- and 4d-transition metals which form mixed hydridocarbonyl or/and hydridocyanocomplexes at redox potentials which can be achieved in living matter, namely Cr, Mo, Mn, Fe, Co, but neither Ni nor Cu. Such stability in living matter means the hydridocarbonyl(heteroligand)metallates to be stable towards water at least for short periods of time and not requiring reductants such as sodium naphthalenide, alkali hydrides or AlH_4^- for preparation (this also excludes "extremely reduced" forms of these and other metals like the protonated Ellis salts, e.g. $[HV(CO)_5]^{2-}$). In addition, cyanide is one of the co-ligands known to considerably increase thermal stability – and reduce acidity (Fränzle 1992) – of carbonylhydridometallates of the said metals, much like PF_3 (Kruck and Lang 1997), phosphines or arsines do. Zn can promote hydride transfer from and to sugars and other aldehydes also while neither Zn carbonyls nor hydridocarbonyls of whatever kind are known. Now inserting the above Eq. 2.17 into Eq. 2.4, taking account of slight differences of the regression equations of the kind of Eq. 2.4 one obtains as limiting criteria for the two gas oxidations of dihydrogen/hydride and of CO:

Hydride: $c_{1d} - 0.30\ x_{1d} \geq 5$ and

CO: $c_{1d(neutral)} + 0.99\ x_{1d(neutral)} \geq 5$,

while for reduction of water by hydrogenases [S] ≈ 55 M/L and thus

H_2O: $c_{1d(neutral)} + 0.04\ x_{1d(neutral)} \geq -1$,

which yields the forthcoming Table 2.10 for the three interlinked reactions

Using data of c- and x-parameters for different oxidation states of one given element for a first orientation (Fe, Ce, Tl, V), Table 2.10 shows that interactions with metal ions (forming hydridometallate intermediates) will bring about hydrogen oxidation when 3d-ions in higher oxidation states are involved, whereas low oxidation states support reduction of water. However, an oxidation of CO cannot be catalyzed by these ions in their usual oxidation states: Oxidation of coordinated CO in aqueous media takes place by Hieber`s base reaction, beginning with addition of hydroxide ion to the C atom of carbonyl CO. After subsequent cleavage of CO_2 from the α-metallocarboxylate the electron pair remains with the metal ion, forming products such as $[Fe(CO)_4]^{2-}$ (from iron(0) pentacarbonyl), and may be transferred from there to oxidants or organic electrophiles. Though metal carbonyl species in high oxidation states like Ir(III), Ru(III), Os(IV) or Pt(IV) often readily decompose

Table 2.10 c, x and reactivity of 3d metal ions towards CO, hydride and water

M	V(II)	Cr(III)	Mn(II)	Fe(III)	Co(II)	Zn(II)
c_{1d}	0.45	0.64	0.3	−2.45	1.17	−0.29
$c_{1d(neut)}$ [a]	3.70	3.90	3.53	+0.57	4.47	2.90
x_{1d}	−12	−15.4	−5.2	−22.42	−5.3	−8.93
$x_{1d(neut)}$ [b]	−17.6	−21.7	−9.2	−30.4	−9.3	−13.8
$c_{1d(neut)} + 0.99x_{1d(neut)}$	−13.7	−17.6	−5.6	−29.5	−4.7	−10.8
$c_{1d} - 0.30x_{1d}$	4.05	5.26	1.86	4.28	2.76	2.39
$c_{1d(neut)} + 0.04x_{1d(neut)}$	3.00	3.03	3.16	−0.66	4.10	2.47

[a] A separate regression was derived for uncharged monodentate ligands like ammonia, pyridine, nitriles or dialkyl sulfides, DMSO

[b] $[Co(CN)_5]^{3-}$ spontaneously reacts with water to afford the hydridocomplex $[HCo(CN)_5]^{3-}$ and the corresponding hydroxo-species (Cotton and Wilkinson 1981) which yields the following values with $c_{1d} = 1.50$ and $x_{1d} = +2.63$ for the terminal coordination step (sixth ligand): $c_{1d(neutral)} + 0.04\ x_{1d(neutral)} = 4.82 + 0.36 = 5.18$. For other Co(II)complexes, spontaneous oxidative addition of water (protons) or of thiols (cleavage of the H-SR bond) – and of dioxygen – is also known. Mixed-ligand hydridocobaltates(III) can then release molecular hydrogen by either photolysis (Hennig and Ritter 1995) or acid attack in aqueous media (Fränzle 1992), they rather directly oxidize the solvent [4] or solvolytically replace CO ligands (also Willner and Aubke 2002) rather than oxidize the latter bound CO. Ni carbonyls form clusters when subjected to conditions of the base reaction whereas $[Mo(CO)_6]$ and derivatives thereof undergo it – forming $[Mo(CO)_5]^{2-}$ or $[Mo(CO)_3L^2]^{2-}$ – only under rather drastic reactions (Mo hexacarbonyl is formed under ambient conditions by non-identified microorganisms in landfills also (Feldmann 1999)). Keep in mind that CO oxidases in anaerobic bacteria and archaea generally contain nickel while CO oxidation in aerobes is effected by molybdopterin units in corresponding enzymes (Kaim and Schwederski 1993).

Release of H_2 from water might be effected by quite a number of different 3d-ions via the hydridocomplexes; although CO and cyanide appear to be most simple and thus "primitive" ligands, it remains doubtful whether these enzymes are as "archaic" as often purported, given both the complexity of cyanide biosynthesis pathways in plants or fungi (Castric 1977; Gleadow et al. 2003) or arthropods and the difficulties which arise when substantial amounts of CO are assumed in the prebiotic atmosphere (cp. Chapter 5).

There is yet another biochemical reaction involving CO (apart from its role as a transmitter substance in animals) which should be addressed here: CO is activated and linked to some Ni-CH_3 moiety in a [Ni(deprotonated oligopeptide)(CH_3)] complex attached to a Ni or Cu carbonylpolythiolate complex which affords acetyl-CoA via some Ni acetyl system by CO shift and insertion in a manner very similar to the Monsanto process for production of acetic acid from CO and methanol. The latter affords methyl groups attached to the metal (Ni here, Ru in the original Monsanto process). This transformation takes place in a clostridium, namely *Moorella thermoacetica*.

2.2.6.6 Metals in Catalytic Chemistry and in Biochemistry

For obvious reasons, it was thoroughly investigated in technical and synthetic chemistry which metal ions (or complexes or solid compounds thereof, e.g. MnO_2,

[4] For example, from Lever`s formalism it can be directly calculated that Ru(II/III) redox potentials for derivatives formed by neutral ligand attack on the chlorobridges in ($[Ru(CO)_3Cl_2]_2$) are similar to that of molecular fluorine (!).

CeO_2 as oxidation catalysts or $[Rh(CO)_2HalI]^-$ in acetic acid synthesis from methanol and CO) are most useful (efficient and durable) for catalyzing some chemical process (for overviews on this issue, e.g. Ochiai 1968; Jolly 1976; Riedel 2004). At this very stage of argument, numerous "mismatches" between biochemistry – which is a result of evolution approaching **local** optima in an event space amidst a plethora of limiting conditions (Rechenberg 1973) – and results from empirical lab-scale or technical chemistry show up. For example, it has been known for 70 years that transport of dioxygen by reversible binding to metal ion-based carriers (rather than physical dissolution in a liquid like water, oil, or some perfluorinated amine or hydrocarbon) is best accomplished by using complexes of Co^{2+} with amines, amino acids or oximes, including proteinogenic ligands like glycinate or asparaginate (McLendon and Martell 1976; McLendon et al. 1976) and dihydrogen may be transferred as a reductant to organics (omitting PGMs) by Co complexes ($[Co(CN)_5]^{3-}$; cobaloximes) likewise (Schrauzer 1976) while biological systems use other metals for the same task:

- Fe or Cu (arthropods, molluscs) for dioxygen transport even though irreversible oxidation can be suppressed by strong sterical congestion only in these systems, and
- Fe and/or Ni in hydrogenases

Thus, while biocatalysis can speed up chemical transformations by more than 10 powers of 10 (notwithstanding reactions which would not occur spontaneously at all, such as N_2 reduction) (Wolfenden and Snider 2001), this sometimes is accomplished off the theoretical optimum. It remains to be seen whether this is due to constrictions of reaction conditions (obviously not applying to O_2 transport or H_2 activations) in biological materials or a result of the metals being bound to polymers, or just a kind of "historical compromise" in evolution. For example, dioxygen transport – for removing it from sensitive (parts of) cells or transporting them inward as an oxidant did not become an issue earlier than 2.3 bio. years BP. Although simple amino acids produce quite efficient and sturdy O_2-carrying Co(II) complexes, by then sophisticated proteins had evolved rather than doing biochemistry on catalysis/transport simple metal complexes anymore. However, as shown by integration of a porphyrine ring directly attached to Fe(II) in haemoglobine (unlike the simpler ligand spheres of haemocyanin (imidazols only) or haemerythrine (carboxylate and hydroxo bridges)) additional developments were required in the systems then used which would not have been necessary at all if using cobalt instead. As Co now is essential for quite a manifold of organisms (animals, protists, many kinds of N_2-assimilating bacteria) which evolved long ago, it is unlikely that it was unavailable for global geochemical conditions 2.3 bio. years ago. Thus, a list (Table 1.1) of such "discrepancies" is needed.

The amount of some element required to meet the various functions and the number of different ones is not easy to estimate even if results from elementary analysis are given: roughly speaking, there is both a "low-metal" and a "high-metal" pathway of biochemistry, with small amounts of Mg sufficient for photosynthesis including CO_2 fixation in both plants and chemolithoautotrophic bacteria whilst heterotrophy takes higher amounts, especially in fungi and certain kinds of bacteria, not to mention iron bacteria, etc.

Some of the functions mentioned before can be accomplished with minute amounts of metal, including certain catalytic functions, others take large amounts by their very nature, such as the osmotic functions of Na, K and their gegenions. Accordingly, species and taxa which are more complicated with respect to their biochemical networks – e.g. autotrophic organisms, including chemolithoautotrophic bacteria, which can/must create a manifold of compounds almost from scratch (CO_2, NO_3^- and other equally simple precursors) rather than extracting them from food, as animals or fungi do – need not require some larger number of different essential elements – rather, the contrary holds true (≈16 essent. elements in higher plants vs about 20 in animals or macrofungi) – nor larger amounts of these micronutrients (cp. Fränzle and Markert 2002b).

2.2.7 The Electrochemical Ligand Parameter, Metal Affinities and Chemical Ecology

With respect to metal ions, ecological stoichiometry is much in its beginnings still (one reason to extend this volume to that topic as suggested by one referee), giving just sketches of what happens to metals in a trophic chain (ibid. for limnetic systems). Thus, there is no

relationship whatsoever between abundance of some element inside an organism and essentiality, and this renders attempts to link environmental abundances or BCF values to essentiality or even number of functions (Kaim and Schwederski 1993) futile. Even less tenable is the idea (advanced, e.g. by Egami 1974) that assumption of some biological functions – and thus essentiality – might be due to a particular abundance of that element in the environment or there might even be some lower limit of environmental (e.g. ocean water) concentrations to have essentiality. Moreover, living beings are composed of thousands of different chemical components – although they principally share certain identical monomer parts such as 22 proteinogenic amino acids or five nucleotide bases, a couple of sugars and ≥7 essential trace metals – yet sequestration, transport and biochemical integration of some trace or ultratrace element may well be effected by some selective compounds produced in rather small amounts (possibly even lower than that of its substrates), too. In some mushrooms of genus *Amanita* there is a N-hydroxyiminodicarboxylic acid which selectively sequesters V(IV) (Rehder 1991), with the $[VL_2]$-complex formed called amavadine, to substantial concentrations: total V, most of it bound to amavadine, is 180 mg/kg DM in *A.muscaria* (Rehder 1991; Lepp et al. 1987), vs a few 100 µg/kg in most green plants (Markert 1996; Kabata-Pendias and Pendias 1984; Emsley 2001). There are various catalytic properties of amavadine (peroxidative halogenation, hydroxylation of arenes and alkanes (Reis et al. 2000) or direct formation of acetate from CH_4, CO and oxidant), while V functions as an oxidation catalyst in these fungi could not be attributed to any other (e.g. vanadoprotein) species so far in spite of an established essentiality (Lepp et al. 1987). Hence it is conceivable at least that amavadine is (also) a biocatalyst rather than simply a sequestering agent or storage form for vanadium. However, as a rule, the fairly simple and "open" (thus not sterically contriving) chelating sites of carrier proteins like transferrines (in animals, bacteria) display poor selectivity with regards to metal ions which they can transport (Duffield and Taylor 1987). However, here is remarkable selectivity in other respects (Duffield and Taylor 1987): transferrins do react with only some of the REEs.

Hence the problem has to be adressed from a perspective of bioinorganic chemistry rather than from analytics: we are posed to ask which chemical features are in favour or disfavour (of) a catalytic function and how to cast these into numerical, quantitative terms. Some elements which are famous for their versatility and efficiency in both homogeneous, heterogeneous (including organometallic) catalytic chemistry, like the platinum-group metals (PGM) are not at all used in biology. For identifying suitable (metal-based) biocatalysts among the more common metals, by theoretical considerations as well as by simple comparison with procedures/methods of technical catalytic chemistry done on the same substrates (table 1.1), some general criteria hold:

- The chemical transformation must take place by reversible interaction between catalyst and substrate, leaving the former untouched after cleavage of the substrate which should occur rather spontaneously (labile binding).
- The metal-bearing center which catalyzes a transformation must be stable under biological conditions or be activated by simple redox transformations (say Fe or Ni porphyrines although neither the $FeO^{2+/3+}$ or Ni(I) forms are really stable in water).
- There must be no side reactions (in aqueous or lipid phases, respectively) which destroy either the very protein matrix of the enzyme or outer biologically important components of the organism even though intermediates of the catalytic cycle might be highly reactive, like O_2^-, H_2O_2, $HalO^-$, $FeO^{2+/3+}$, Ni(III) or Cu(III).

In aerobic organisms formation of these intermediates may even contrive the use of metal ions hitherto selected during evolution for their catalytic properties: even metal ions which are completely redox-inert in biological conditions like Cd^{2+} or Pb^{2+}, might undergo bioalkylation at most, can bring about "oxidative stress" by reactions with lipides, like many organic compounds do, producing O_2^- (superoxide) radicals or H_2O_2 or alkylperoxy radicals also without an electron transfer by themselves (Karnovsky 1994; Nieboer et al. 1986). Catalytic selectivity and versatility do not suffice as criteria for a possible biochemical "usefulness", in addition unwanted side reactions of these and similar kinds must be limited. On one hand, some very abundant elements which yet are not used in catalyzing biochemical transformation (Al, Ti) probably are simply not versatile (capable of promoting a sufficient number of different reactions) enough in aqueous medium (Hoagland's nutrient solution to be added). Knop's one does contain Al, Ti, Li (and Co) besides

those metals known to be essential for support of plant life but their mere addition of course falls far short of any proof of essentiality.

As signal conduction and Na/K antagonism do play no larger rules in plants (except for few "sensitive" plants like venus flytrap or mimosa or halophytes which can sustain only if and as long as capable of restricting Na concentrations inside their cells, hence depend on Na/K ratios in the surroundings), and aspects of osmoregulation and constructing/fortifying structures are likewise set aside to focus on issues of biocatalysis as brought about by essential element speciation forms. For example, Sr will be obviously required by those marine algae which construct exoskeleta from $SrSO_4$, with no alternatives, as will be Si for making SiO_2-based fortifying fibres or needles. These cases represent some kind of Sr or Si essentiality in plants but are not going to be addressed here even though they take some selective "handling" of Sr or Si, presumably by means of corresponding carrier proteins. On the other hand, Ba is known to be required by Desmidiaceae (algae) but will not be integrated into some mineral structures by them.

2.2.7.1 Speciation of Metals Before and During Root Uptake by Plants

Speciation forms of most trace metals (except the alkali metals) in soils will depend on geochemistry while that of Mo – unless in fully wet, reducing soils – will probably be $[MoO_4]^{2-}$, transporting it like borate with the sulfate ion transporter system. The other metals will be influenced by both geochemistry and the ligands delivered by roots or fungal mycelia to the soil. Whether citrate or malate (plants) or N donors (hydroxamates, amino acids; fungi) will coordinate metals in the soil or ground water depends on the stability of other species already trapped (solid mineral phases, complexes with polymeric 1,2-diphenols (humic acids), carbonate, etc.) and thus on pH, redox potential and biological activity with the soil liquid. So, Strasburger and Sitte (1991) obviously oversimplify the situation in a manner negative to understanding when they state "the essential metals other than Mo are taken from the environment as cations".

More realistic, while and because admitting that there are still hypothetical steps and binding partners in their transport model, is the description of transport and bioaccumulation of metals given by Clemens et al. (2002) (Fig. 2.6). Figure 2.6 shows that and how a transformation of ambient speciation forms takes place already outside the plant organisms by way of ligands delivered into the rhizosphere (root exsudate). There are several elements which may produce oxo- or thioanions depending on redox state and pH of ground water in various oxidation states, e.g. Cr, V and U. Chromate(VI) and vanadate(V) are readily taken up by plants but – e.g. in soybean or radish plants – undergo reduction to afford V(IV) and Cr(III), respectively, already within the roots. Therefore, only some small part of these metals does make it into the parts above the ground because complexes are too weak (Rehder 1991) to interact efficiently with other carriers after reduction and before admission to the xylem. Concerning Cr(III), one must not (mis-)take the kinetic inertness of many complexes for high thermodynamic stability: they are not. In addition, there is co-precipitation of Cr as a phosphate with Al(III), Fe(III) reducing xylem transmission furthermore.

Two different metal ions may be compared to each other using c, x and the electrochemical ligand parameter $E_L(L)$ to compare complex stabilities for both ions linked to the same ligand (in the same manner). Like Ahrland et al. showed metal A to yield a more stable complex with a ligand 1 but metal B with ligand 2 (different binding preferences), the same may happen if A and B get into contact with biomasses of different taxonomical species or just different organs. The outcome will be fractionation of A and B (and the multitude of other metal ions) between these species, with a third taxonomical species likely to provide yet another pattern of fractionation (Markert (1996) discussed a total of 13, Garten (1976, 1978) even many more). From this, low correlation coefficients among abundances of A and B in a multitude of species are likely to result. Comparing abundances (or, more precisely, bioconcentration factors) among different species, thereby obtaining inter alia a correlation coefficient for the abundances of some pair of elements (which need not be metals) thus gives information on complexation properties of the corresponding biogenic materials: thus, average binding properties are measured. Except for some commercially purchased reference materials, Markert obtained his set of plants all from the same site (Grasmoor bog near Osnabrück, Lower Saxony, FR Germany, Fig. 2.22); accordingly soil background levels of the metals should be similar so that there is a linear relation between metal concentrations measured in photosynthetic organs and BCF values, at least as a first approximation. Like some

chemically homogeneous ligand is distinguished by a certain electrochemical ligand parameter (unless it does form linkage isomers, of course), the fractionation behavior can be described by an effective electrochemical ligand parameter defined and calculated by rearranging Eq. 2.4 in order to extract $E_L(L)$ as follows:

$$-\log k_{diss} = x_{nd} \times [E_L(L)] + c_{nd} \quad (2.4)$$

gives

$$E_L(L) = \{-\log k_{diss} - c_{nd}\}/x_{nd} \quad (2.10)$$

This effective electrochemical ligand parameter will pertain to the biomass of some species, at least to some organ such as a plant leaf as well as to coordination-chemical properties of dead biomasses such as marine POM. The data is extracted from abundance distributions of elements with respect to their ecochemical backgrounds, that is, from BCF values (most straightforwardly for aquatic plants and animals). Regressions to determine c and x from Eq. 2.4 were done by using data collections on complexation constants (mainly Furia 1972; Mizerski 1997; Izatt et al. 1971) and a list on electrochemical ligand parameters (Lever 1990).

Biomass and fluids contain substantial quantities of potential ligands with which most heavy and some light (Mg, Be, Ti, Al, Y) metals form rather stable complexes. Thus, uptake, state (speciation) and partition including "dumping" sites (removal as leaf or needle litter) of metals in plant biomass are mainly controlled by features of their coordination chemistry. Biomass contains various different ligand sites, including carboxylate, amino-, imidazol- or polyphosphate moieties retained by polymers with mostly covalent backbones. **Accordingly, any causal account for the Biological System of Elements must refer to coordination-chemical properties of biological ligands**.

As a rule, aquatic organisms (plants) derive their demands, e.g. of metal ions directly from the solution. Thus, the argument on BCF/complex stability relationships fully holds for terrestrial organisms only. Yet, there are hints which suggest that both algae, bacterial biofilms did hold foot on solid land far earlier than hitherto assumed, that is, much before large amounts of dioxygen cumulated in the atmosphere, only then allowing for substantial nitrification by either lightning or biochemistry. Thus nitrate and in turn hydroxamates were unlikely to be used before the onset of the Paleozoic.

Whatever be the reason for the Eq. 2.21 to apply, the relationship can be used to analyze some feature of **metabolic thermodynamics**, namely, the minimum amount/share of nitrogen which is retained in some organ when releasing ligands to soil directly. This minimum value of N/C or **maximum of C/N** in respective organisms does exist because the values from which Eq. 2.21 was derived are minimum redox potentials at which the required oxidant is no longer detectable. Accordingly, delivery of substantial amounts of the sequestering ligands in Table 2.2 requires the ambient redox potential to be higher and thus, C/N to be smaller notwithstanding possible differences of C/N between subterraneous organs of some organism and its total composition. Except for thick old roots which do no longer substantially contribute to element uptakes from soil but rather behave as a kind of mechanical anchor and the C/N ratios (about 200) of which resemble that of other kinds of wood (Sterner and Elser 2002), the latter differences are but fairly small. Notably, this argument does not depend on any assumption concerning the manner of sequestration of any metal ions required to make these sequestering ligands, e.g. that of Mo contained and active in nitrate reductase. In addition, but some of the mentioned ligands contain nitrogen at all, leaving aside, e.g. hydroxicarboxylates or 1,2-diphenols, hence there is no direct coupling other than that from metabolism.

Optimum availability of some metal in turn depends on the differences among electrochemical ligand parameters of N-containing and N-free ligands present in soil. Thus, given a couple of such (different) ligands can be produced at sufficiently high redox potentials, availability of specific metals (including some of little use to both plants and fungi, such as Cd) will peak somewhere (Fränzle 2008). This might shape an ecosystem, especially if it gets full circle: soil-contacted organs of different creatures – including mykorrhiza – will then "cooperate" to stabilize extraction of the set of metals needed to produce sufficient amounts of sequestration ligands as well as permitting biochemistries including large amounts of N. For example, Fe(III) can be extracted from an oxidizing soil by means of diphenols or hydroxamates or combinations thereof. For Fe(III), $x_{2d} = 21.39$, with a difference of $E_L(L)$ between diphenols and hydroxamates of +0.21 V. Then, C/N_{red} (reduced denotes the numbers/molar concentrations of the corresponding ligands; that is, corrected for the intraligand C/N ratio) should be very large in soil. Anyway, it will work only with both nitrate and dioxygen present in soil.

Roots (or mycelia) tend to deliver ligands themselves to the soil which bind metal ions from soil solution

Fig. 2.5 *Malcolmia maritima*, the color of flower unaltered by metal exposition. Concerning isolated anthocyanes (probably oenin) from grape (*Vitis vinifera*), the red pigment does not apparently react with Fe(III) in either water or ethanol but turns dark-purple with Cu(II) and yields a blue precipitate with Pb(II) acetate (Anderson 1924). It also behaves as a pH indicator. The yellowish-green colour observed in live plants thus can be due to superposition of some chlorosis and formation of blue to blue-red (i.e. purple) complexes; possibly some third ligand such as an amino acid also contributes

or even mineral/clay phases around and render them better accessible to the plant or fungus. Like soil solutions, hydroponic culture solutions contain chelators and other ligands, making speciation of dissolved metals too complicated to be grasped other than by hydrogeochemical models like MINTEQ+. Often complexation reactions yield intensely coloured complexes (Riedel et al. 2004); this also can happen when metal ions penetrate into biological matter, e.g. causing flowers to change colour if the plants are grown on metal-rich soils (Strasburger and Sitte 1991). For example, in the brassicacea *Malcolmia maritima* (Virginia stock) there are small amounts of red anthocyanes dyeing the flowers light rose to bluish (Fig. 2.5), but in presence of Cu, Zn or Pb their colors change to yellowish-green upon M^{2+} complexation. This change of flower colors is practically used in ore prospection.

2.2.7.2 Further Transport of Metal Ions Inside Plants

For any of these organisms, delivery of ligands to soil or groundwater which then sequester metal ions (both essential and non-essential) requires decoupling of matter from some metabolic cycle. In simple cases, like with green plants, there is no demand for additional reactants to produce the corresponding ligands, as citrate or malate are taken from the tricarboxylate cycle directly. More generally speaking, however, formation of such extracellularly active ligands takes additional reactants which modify some precursor to obtain the moieties which in turn can coordinate some metal ion (e.g. phenolate, hydroxamate, amino groups), with the complex thus formed eventually being reabsorbed by the organism which emitted it for purposes of its own metal supplies. Fairly often, the additional reactant is an oxidant, e.g. dioxygen is needed to convert aromatics or monophenols into oligophenols, while NO or nitrate are required for making hydroxamates from 2-ketoenolates (hydroxylamine NH_2OH which cleaves esters by direct nucleophilic substitution to yield hydroxamates, is not normally an intermediate of either biological NH_3 oxidation or NO_x^-, N_2 reductions. Apart from this, the hydroxamates which are produced by fungi or soil bacteria bear an alkyl or aryl group at N, accordingly would have to originate from N-functionalized hydroxylamines which are rare in biochemistry, also).

Citrate (or malate) is shuttled back into the tricarboxylate cycle once the corresponding complexes have been resorbed by the plant roots; accordingly the complexes dissociate in a kind of oxidative ligand exchange, giving way to formation of new complexes of the absorbed metal ions combining with chelators in the cytosol. The equilibrium is shifted to the left by removal of the original ligand which had effected resorption. By evaporation in leaves a vertical water current is produced which also transports metal complexes through the shoot (stem) axis upward; thereafter the metal ions will get bound to nitrogeneous ligands like peptides and proteins. Metal transporters like the protein IRT-1 (IRT = iron regulated transporter) occur in epidermis cells next to the rhizosphere of plants and in the root/shoot interfacial region, e.g. in wheat or root epidermis cells of *Arabidopsis thaliana* (Clemens et al. 2002). Like in albumins of animals, IRT-1 effects metal binding by four histidine residues (i.e., imidazol ligands). In the former albumins metal ions such as Ni^{2+} are known to be rapidly removed from the protein sites (a matter of minutes at most) by simple addition of amino acids (Tabata and Sarkar 1992) which means $k_{ass} < 10^6$ for nickel ions; accordingly the transporter is a rather poor ligand.

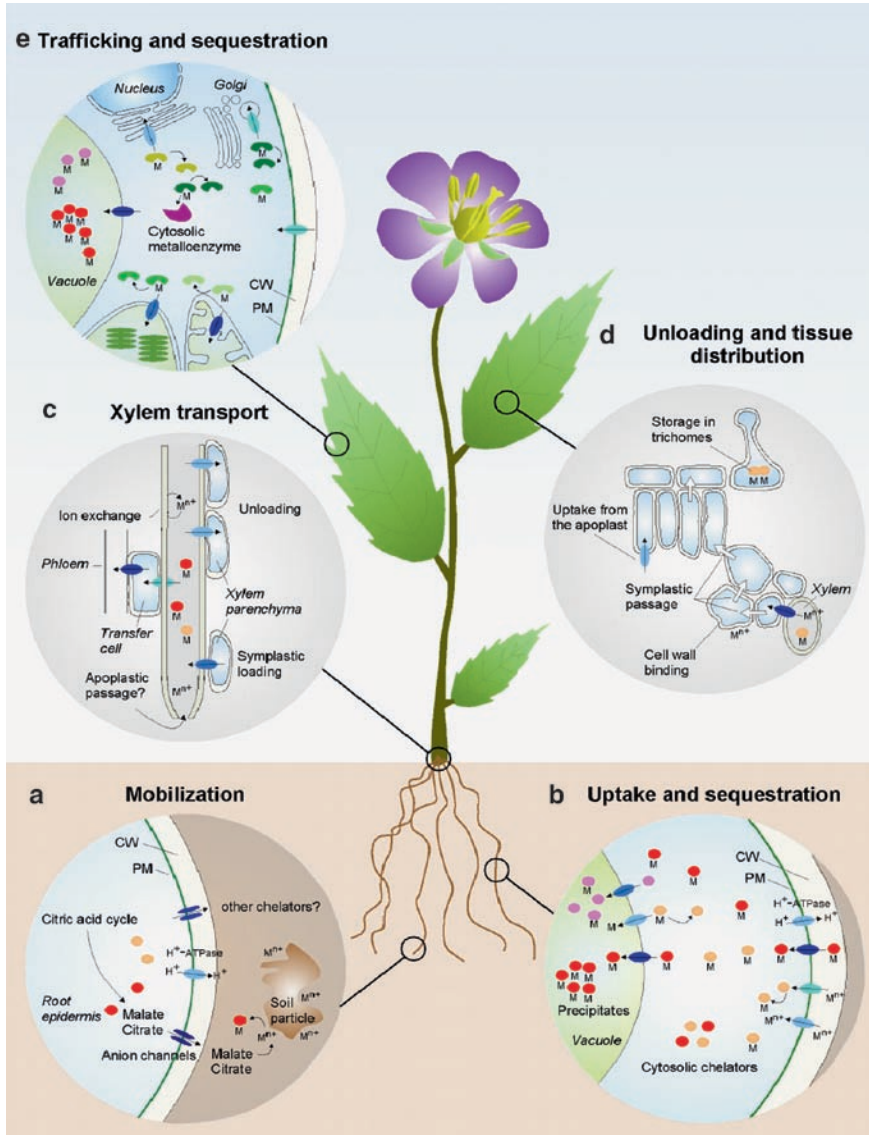


Fig. 2.6 Molecular mechanisms hold to explain accumulation of transition metal ions by and in plants. Letters (**a**) to (**e**) are to be taken in the same vertical arrangement in both plant and this picture, e.g. a = mobilization around the root, c = transport within the xylem. (**a**) metal ions get mobilized by secretion of chelators which in addition acidify the rhizosphere. (**b**) uptake of hydrated metal ions or (rather) their chelate complexes is augmented by various systems bound to the plasma membrane. (**c**) transport of transition metals from roots to shoot occurs via the xylem. Presumably the larger share is transported by means of the root symplast; an apoplastic passage in the root tips is also conceivable. After exchange (oxidative destruction) of the original ligands metals which made it into the xylem are other kinds of chelator complexes or else aquated ions. (**d**) After getting into the leaf apoplast several metals are bound to the various kinds of leaf cells and move among cells by means of the plasmodesmata. (**e**) Uptake into leaf cells is catalyzed by various carriers (not displayed). Trafficking of essential elements inside cells is effected by specialized, selective transporter proteins (metallochaperons) and carriers attached to endomembranes (*nota bene*: these latter processes occur in all cells rather than in leaves only). Symbols and abbreviations: CW = cell wall; M = metal; filled circles = chelators, filled oval dots = transporters, bean-shaped symbols = metallochaperons. Cp. Fig. 2.16 for transformation into a description focussed on subsequent biochemical changes of speciation, cp. Fig. 2.9 for the different manners in which fungi, mosses and vascular green plants use chemical elements and what they do to sequester them. Reproduced with permission by the author (from Clemens et al. 2002)

For selectivity of binding of metal ions, binding to carriers and its selectivity are relevant. Some ligands, production rates of which may be increased upon metal exposition, like nicotianamine (Fe, Cu), histidine (Ni), or citrate (Cd, Al) increase carryover of the said metal ions into the xylem in both hyperaccumulators and "normal" plants as do chelators which are not altered by metabolism, e.g. EDTA, when they are added to the soil. There is a difference in structure: whereas in albumines four histidines (or two histidines and two carboxamides) are located at one end of the peptide sequence, they form a bridge between two helices in IRT-1 (Grossoehme et al. 2005). The limited complex formation stabilities of these ligand site arrays, allowing for reversible binding rather than the role of some "cation trap", is just suited for transportations tasks. Amino acids may "extract" the metal ions from this carrier (also others than Ni) by forming more stable complexes as is done in isolation of demetalated apoproteins by EDTA addition (Noertemann 1999).

At low ligand concentrations, there will be an equilibrium among chelate complexes and aqua- or hydroxoions in the xylem. Usually, pH ≈ 6 in the xylem; thus many aquaions get deprotonated including some forming (but weak) complexes (e.g., VO^{2+}). Owing to free phosphate ($H_2PO_4^-$) levels of up to several mmol/L, mobility of metals in the phloem likewise may differ markedly, being lowest of course for those metal ions which form hardly soluble phosphates (Li, Ca, Sr, Ba, Pb, Ag, etc.) and are immobile in the phloem, mobility being but slightly better for Mo, Fe, Mn, Co, Cu or Zn; conversely heavier alkali metals Na – Cs or Mg are pretty mobile there (Marschner 1986). Also sites where metal ions are located can differ in higher plants: some occur outside the cells mainly, becoming active parts of enzymes out there also (Ca, Cu, Al, [Mo]) while others reside and catalyze some processes either inside the cell (Mg, Fe, Co, Zn, Ni or Mn) or in cytoplasm (Mg, Co, Zn (Williams 1986)), being accompanied by several non-metals other than the ligation partners in each case (Si, Cl or B).

By relocating them via the phloem, plants can recover highly mobile ions such as K or Mg from e.g. aged leaves before these are dropped into the litter. This mechanism hampers a direct comparison of metal concentrations and distributions among different plant species (as was done before by correlation analysis (Markert 1996)) as transport or binding properties are likely to differ also. Apart from this, some elements less similar to each other than the REEs are also distinguished by very highly positive correlation coefficients of their abundances in plants. Accordingly, pathways of transport and accumulation are likely to be very similar among these organisms as well. This prompted an attempt to quantify differences among the various plants, too (cp. Sections 3.2 and 4.2).

As a rule, green plants employ chemically less "sophisticated" species to take up metal ions via the roots than animals, fungi (e.g., yeasts) or bacteria do. In addition, plants change their environments and speciation forms of metal ions which occur there less severely than both animals and chemolithoautotrophic bacteria do by both acidic leaching (stomach, *Thiobacillus*) and production/delivery/use of highly effective sequestering agents. Either process does not only change the environmental speciation thoroughly, with various soil organisms even being able to cause irreversible changes to the metal ion retention capacity of soils by complexation to polymers or in solid mineral phases (fungi, earth worms). When compared to this, metal distribution, partition and accumulation in plants should be more straightforward to understand, with the corresponding models being less complex than those applied for bioaccumulation in animals (cp. Paquin et al. 2003). Often alkaloids which are active in metal ion enrichment also pile up in photosynthetic organs for some period of time, e.g. the xanthine compounds caffeine, theobromine (Habermehl et al. 2003; Colacio-Rodriguez et al. 1983) and associated polyphenols such as caffeic acid in young leaves (leaf-tips) of tea (*Camelia sinensis*) or mate (*Ilex paraguayensis*). In older leaves concentrations get smaller, obviously in favour of releasing metals which were transported to the ultimate parts of a plant again while these parts get into using the metals. Of course, these and similar alkaloids, polyphenols are useful for accumulation of metal ions during plant development also, hence there may be stockpiles in seeds, e.g. caffeine (and caffeic acid) in coffee beans. Concerning cyanide, there is both production of cyanide (usually from glycine precursor) and conversion into other ligands (asparagine via 3-cyanoalanine) in small roots (Ting and Zschoche 1970), contributing to chemistry of metal uptake. As the nitrile 3-cyanoalanine and carboxamide asparagine are formed from cysteinate, there also is a considerable increase in $E_L(L)$ of the third (terminal) donor site of the amino acid, changing selectivities in a similar way like cyanide does.

A look aside to other groups of elements and organisms: the realm of **chemical ecology** and food chain shaping.

Substantial enrichments of radionuclides are known to occur with aquatic plants (Nuclear Task Force 1996; Weltje 2003), and REE ions which usually have highly negative x_{1d} and also x_{2d} values (even being rather identical for Y(III), Ce(III)) get thoroughly enriched there. For distributions of cerium (^{141}Ce autoradiography) in a terrestrial plant see Guo et al. 2007. This tendency for pronounced metal enrichment of course extends to organisms which even use metals as electron sinks or sources of metabolism, like Fe-oxidizing bacteria *Leptothrix ochracea* and implies binding partners to have $E_L(L) << 0$ V (cp. Figs. 2.1 and 2.9). For *L.ochracea*, $E_L(L)_{eff} = -0.16$ V for a total analysis including biogenic oxide concretions.

There are several methods for directly depicting metal distributions in plants, including autoradiography and X-ray fluorescence (XRF or XFS). The former method – essentially the procedure by which Becquerel discovered the phenomenon of radioactivity in 1896 – means using and detecting (by photographical means) emission by some radionuclide (often non-metals such as ^{11}C, ^{14}C, ^{32}P but also $^{55;59}$Fe and ^{109}Cd (Figs. 2.7 and 2.8) in some instances) which was administered to the plant before. This will produce a quantitative picture of the spatial distribution of radiation emitted within the organism or in another kind of sample (by darkening of some conventional AgHal-based photographic film) whereas XRF can be done on the untreated plant making use of synchrotron radiation for excitation of stable nuclei also. XRF can also afford – by EXAFS (shifts of typical fluorescence energies) – some information on the ligand sphere around the metal ions; in addition to avoiding radionuclide uses and any other prior sample treatment (but not omitting radiation), it is a multielement method. How different metal distributions can be although there are similar maxima in the upper root region and decreases along the shoot, Figs. 2.7 and 2.8 (the latter by Feller 2005) do show:

2.2.7.3 Equilibrium Models, Concentration Ranges and Biological Functions of Metal Ions

Although equilibrium models will provide realistic representations of distributions of most chemical elements these are unlikely to hold for such metals which both have a number of biochemical functions and bind strongly to the various ligand sites of biomasses, in addition to being rather abundant in the environment (such as Cu). It can be estimated that "free" (i.e., just aquated) Cu^{2+} or Zn^{2+} ions will occur in plant sap at femtomolar or even lower concentrations at best, leaving their transport to coordinated (ligated) species which change ligand spheres several times during this process. There are specialized (metal-specific) transport and storage processes and speciation forms which

(a) Avoid that metal ions strongly coordinating to biomass (like Cu, Zn) will bind to whatever protein or apoprotein around, otherwise putting both its structure and function(s) into jeopardy (becoming thus toxic)
(b) Simultaneously give a reliable supply of these metal ions to enzymes which depend on such ions (say, Cu-dependent oxidases)

Strongly toxic elements like Cd, Hg or U will cause specific repulsion reactions in plants prompting them to

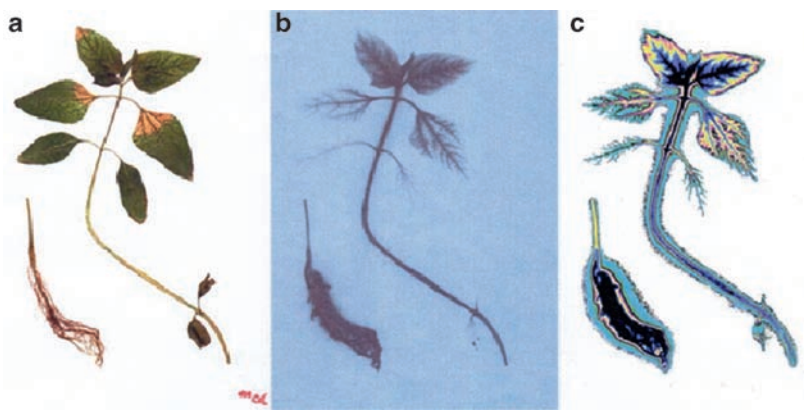


Fig. 2.7 Photograph (*left*), autoradiography (^{109}Cd, $T_{1/2}$ = 453 days) (*center*) and digitally contrast-enhanced version of cadmium distribution in sunflower (*Helianthus annuus*) seedlings (*right*). The root is cut off and laid to the left. Note that there are high Cd concentrations in the vasculae also in the leaves but Cd hardly leaks into the leaves

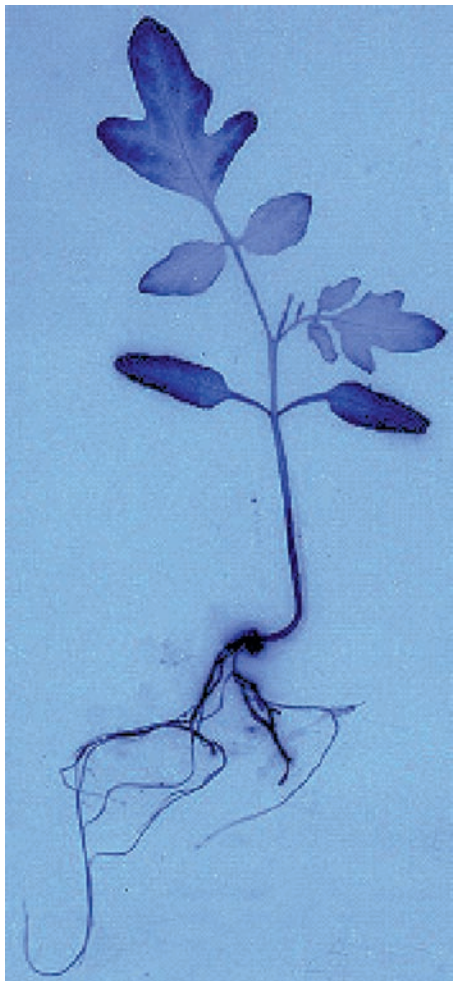


Fig. 2.8 Autoradiography of a young tomato plant; the radionuclide used for obtaining the picture was not specified (Feller 2005). Here, there is some concentration maximum in the upper root also, but the radionuclide does accumulate in the edges/tips of leaves while the vasculae are depleted with respect to radionuclide (reproduced with permission)

produce agents which trap them by chelation (induction of phytochelatins). Accordingly, these data (BCF, intra- and interspecific/organismal distributions) for Cd, Hg or U in plants or animals are more difficult to attribute to "purely chemical" features of transport, because here (and with As, Pb, Ni) there is superposition of

(a) Specific transport/quenching by chaperons (Tottey et al. 2005)
(b) Specific ligand properties in phytochelatins, with, e.g. Cd^{2+} affinity depending sensitively in sequence periodicity there (Dorčak and Kręcel 2003), and – before it gets there – also
(c) Is due to an unlike level of phytochelatin induction by some given heavy metal in different kinds of plant

Thus, there is negative feedback in the environment/metallome system, exactly tantamount to detoxification. For so-called semi-metals some of which also form "colloquial" complexes, like Sb, Bi, Te, the speciation pathway of biomethylation (Thayer 1995) will remove their electrophilic properties altogether, turning the cations into ligands (donors) of their own, whereas with other elements (Ge, Sn, Pb, Pt, Au, Cd or Hg) acceptor properties are substantially altered (see the data (c and x values, Table 2.3) for R_2Sn^{2+}, R_3Sn^{+} and R_3Pb^{+} species) but do not vanish. Of course, redox processes also influence acceptor properties (cp. the data for different oxidation states of V, Fe, Ce or Tl).

To a first approximation, in essential elements the number of different biological functions should correlate positively with the concentration of the corresponding element in biomass, but the amounts which are required to operate biocatalytic processes may be so small that corresponding differences will be undetectable if there is any other – e.g. osmoregulatory – function of that element. Osmoregulation, in particular in halophilic organisms, takes much larger quantities than both catalysis and chemical signalling (alkali metal ions, Ca^{2+}) while no kind of biological function can be inferred from analytic data directly (for example, in humans, there are four or five essential elements with daily requirements ≤1 μmol (total) or ≤1 μg/kg FW, namely Se, V, Co, Mo (presumably +As)).

The corresponding equilibrium state of distributions of various metal ions usually differs from those of the soil substrate, hence entails some fractionation during transport as described before. So, fractionation can be compared to that effected by some single ligand, and Eq. 2.4 and its inversion are then used to define some "effective electrochemical ligand parameter" which describes the capability of some plant organ to fractionate among metals.

Therefore no longer qualitative statements are cast into a numerical framework but easily measurable values are linked to obtain information on complex stability or bond energies. Thus, by linear regression analysis, two new parameters are obtained which – once they are known for sufficiently many different metal ions, both essential and non-essential ones – in turn can be linked to this biochemical property of essentiality. Electrochemical ligand parameters for different complexes of the same metal ion are correlated

with the respective complex formation constants to yield two parameters which are typical of a certain metal ion and way of bonding (denticity of ligand). So the redox potential of some metal complex or the electrochemical ligand parameter of its ligand are taken to determine – and in turn predict for yet other complexes – the complex formation constant $-\log k_{diss}$. The regression Eq. 2.4 obtained in this manner provides some typical (intrinsic) binding stability (axis intercept, c) and a slope (x) which denotes the sensitivity of complex formation towards the (changing) kind of ligand (ligand sensitivity, x) (Fig. 2.1). Conversely, with denticity and electrochemical ligand parameter of ligand being known, a complex formation can be calculated by c and x of the central ion (recall that the electrochemical ligand parameter is obtained by dividing the actual change of redox potential in the Ru(II/III) couples by denticity (Lever 1990)). As a meaningful definition of c and x can only be obtained with sets of a equidentate ligands, the corresponding multiplying factor in the regression equation was skipped for simplicity. The result would be the same except for some alteration of both c and x values in multidentate ligands, but then Eq. 2.4 would be more difficult to handle.

Now, the situation regarding metal fractionation will be discussed for green plants, fungi and animals one after the other, giving rise to trophic networks and eventually geobiochemical cycles, including issues of ecological stoichiometry beyond C, N and P. **Fungi** can only degrade lignin, lignite and other kinds of polymeric phenols if they command suitable laccases, peroxidases, etc. (basidiomycete fungi) and thus require Fe(III), Cu and/or V. As these processes require dioxygen as the initial oxidant, they can only be achieved in an oxic milieu; even corresponding brown coals are considerably oxidized, producing O-rich solids or even oxalic acid. Thus it can be concluded there is Fe(III) with the corresponding c- and x-values (rather than those of Fe(II)) controlling bioavailability when the above ligands are present.

Both fungi and soil bacteria will simply oxidize and degrade substantial parts of soil organic matter to yield CO_2, eventually reducing the ratio $[C/N]_{red}$ to values of 3–4 (Bardgett 2005). During this process, amino acids, hydroxamates and sometimes oxalate are formed. Now let us estimate which metal sequestration strategy, using bidentate agents, must be taken to adapt to $[C/N]_{red} \approx 3.5$ in the soil and $x_{2d} \approx 16$ (causing an enhanced uptake of Fe(III), Cu(II) and V(IV)) for doing such oxidative "crackdown" of phenolic polymers by fungi or other organisms. The result can be obtained from Fig. 2.16: the "best" ligand should have $E_L(L) = -0.03$ V, that is, amino acids could achieve appropriate metal uptake from the sediment pretty well. This and the electrochemical ligand parameters of the N-donors imply values between −0.08 and −0.01 V for the N-free decomposition products of lignines or lignites. By this the "suitable" products of lignin degradation are determined: especially in exocellular processes formation of such products must be avoided which in turn would bind essential metals like Fe or Cu so strongly or in thus hardly soluble forms that retention outside the cell would become too large. The result of such ways into lignin/polyphenol degradation then would be metal ion starvation by the very products of the process. The optimal outcome of these is a product array providing minimum retention which has to be compared to the (hitherto far from complete) information on ligand-active compounds in aged humic substances or in lignins. The latter, as detected by IR- or ^{13}C-NMR spectroscopies, include 3-ketoenolates (higher analogs of acac), 2-alkanoylphenolates, phenolpolycarboxylates, which provide optimum mobilization of the above metals if combined with amino acids or hydroxamates. In soils, amino acids are produced/delivered either directly (grass roots, drying mosses) or produced from protein residues by soil-borne hydrolases while 3-ketoenolates like oxaloacetate are intermediates of the tricarboxylate cycle and provide additional ones upon decarboxylation. It can be concluded that the "strategy" of (basidio- or ascomycete) fungi for lignin degradation is not just the only one which ever evolved but also highly optimized: considering the frequent differences between "biochemistry" and "technical optimum" it is the more conceivable there was just one success as most similar reactions would yield materials from which the exoligninase-forming metals like Mn or Fe could no longer be retrieved.

Thus chemical ecology, coexistence and possibly mutual succession of different organisms which thrive on the same supporting soils are determined mainly by the complex-forming properties of the (chemical, mostly anionic) species delivered by roots or mycelia, respectively. In addition, other kinds of fungi (mykorrhiza) organize interaction and chemical exchanges of both metal ions and N or organic species while biochemical and MnO_2-catalyzed degradations will alter identities, functional (donor) groups, thus $E_L(L)$ and selectivities

of ligands in soil with time. MnO_2 particles are thus efficient if combined with strong oxidants as to render permanganate oxidations even of rather refractory organics like glycine ($\rightarrow HCHO + CO_2 + NH_4^+$) autocatalytic, forming more manganese dioxide (Perez-Benito et al. 1987).

For the "original" species, these reflect the different demands due to different biochemical pathways and catalytic challenges in the various large groups of organisms. Nevertheless, all plants, fungi and soil bacteria can only successfully sequester cations from soil because there is a combination of two features:

(a) They use ligands which are typical for the realm of organisms and do not differ too much in $E_L(L)$.
(b) The c and x values are rather similar except for Cu and Ca among the essential metals; thus the "window of essentiality" (Fig. 3.1) is required for an effective co-existence of soil-dwellers even though they compete for the same resources at first glance: there simply is no option to escape competition by using metals as biocatalysts with very much "deviating" c_{2d}- and x_{2d}-values, no matter how abundant they may be (Al!).

For purposes of theoretical understanding, $E_L(L)$ obviously is the decisive criterion allowing to meet the different demands of green plants and of fungi, also regarding details in soil chemistry.

When feeding by consumption of autotrophs, **heterotrophs** must overcome other chemical obstacles than to balance out (electron retrieval by) water oxidation and CO_2 binding plus reduction which green plants have to do:

- Oxidation of organic matter has to be accomplished in a **selective way**, making use of Zn-, Mo- or Fe-dependent enzymes, leaving behind many different products besides CO_2 and protons.
- Proportional to this oxidation energy can and must be stored by phosphorylation of carboxylate and sugar substrates which is done by Mg or Zn enzymes (kinases, phosphatases) mainly.

N transfer is metal-independent while N oxoanion reduction takes Mo once again. As the stoichiometric composition of the food of second- and higher-order consumers is fairly constant, the relative requirements of the metals which take part in the quantitatively dominant processes in an animal are constant also, and these metals may be acquired from food and from ambient water – or lost to the latter. This can work either way round, depending on the relationship between complex formation constants (and thus, on $E_L(L)$ once again). As shown by Fig. 2.11, this does not hold for phytoplankton: in eutrophic conditions, all C/N- (9 rather than 13), N/P- (<20 rather than 70) and thus eventually C/P ratios (about 150 instead of 900) are decreased. As Mg is implied in both photosynthesis and phosphorylation, C/Mg should not be changed by eutrophification whereas N redox demands increase, and thus C/Mo and Mg/Mo ratios should negatively respond to eutrophification, with an enhanced Mo level in the "eutrophic" alga. As evidenced by the altered CNP balance, (at least many) algae can adapt to this, accordingly must be able to absorb more Mo if exposed to (N; P)-increased conditions. Animals feeding on phytoplankton in turn have more Mo available, then. So it might very well occur that "trying" to keep the necessary stoichiometric ratios among essential metals in an aquatic animal which does feed on something particular needs $E_L(L)_{eff}$ values which bring about thus feeble complexes that certain metals will be lost to water at least if the latter contains humic acids or other kinds of ligand-active DOM. Thus, "translating" the metal ratios of the food to the consumer's own demands given by bioinorganic chemistry, the key topic of (extended) ecological stoichiometry, is a kind of translation which, unlike a given plant or fungus is to thrive on a soil of certain composition, often brings about substantial losses to the environment. Of course, metals like the heavy alkaline earths or Zr which will not appreciably react with humic-type DOM will not undergo corresponding "leaching" from zooplankton.

Concerning zooplankton and larger aquatic animals, it is feasible to calculate some effective $E_L(L)$ in animals in the same way as for plants or bacteria: daphnia (*D.magna*) exhibit a decreasing abundance of metals like above (that is, Fe ≈ Zn > Zr > Cu > Cd > Mn > Cr > Co > V > Mo) while for plant leaves it often is Ca > Mn > Fe ≈ Al > Ba > Cu, in disaccord with both an expected accumulation by the Irving–Williams series giving stabilities of "normal" complexes. It can be seen from Table 2.3 that the "classical" (1953) Irving–Williams series simply corresponds to the sequence of increasing c values for bidentate behaviour: Ba (0.45) ≈ Sr (0.55) < Ca (0.73) < Mn(II) (3.01) < Mg (3.94) < Fe(II) (4.20) < Co(II) (5.48) < Ni (6.65) < Cu (9.04) >> Zn (5.15). Accordingly the Irving–Williams series is observed without any permutations if c dominates in

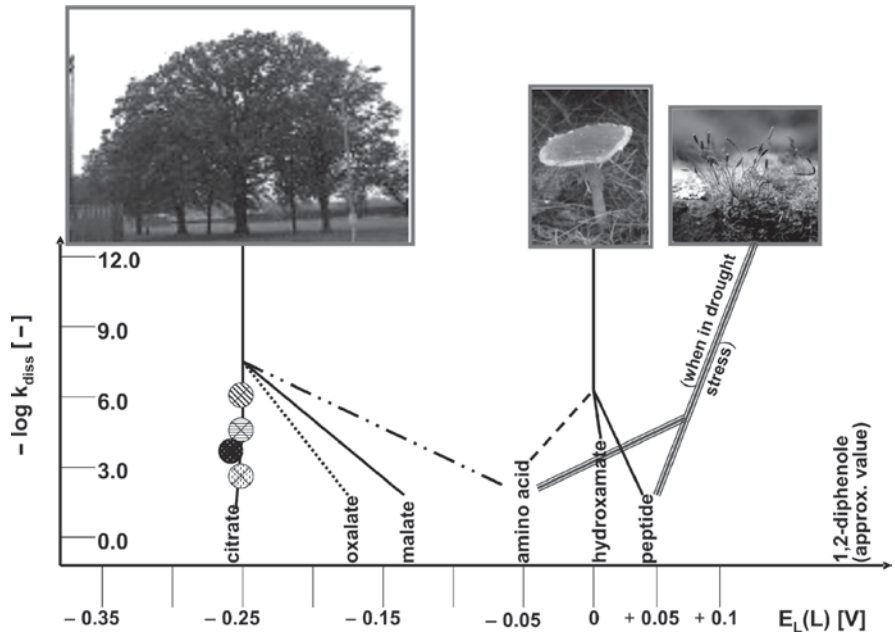


Fig. 2.9 Green plants (*left, represented by beech tree*) and fungi (*right center*) secernate ligands with quite different electrochemical ligand parameters which accordingly display different metal selectivities fulfilling the demands of either heterotrophic or photosynthetic group of organisms. Fungi thus enrich Fe, Cu and V for haem-based peroxidases, laccases and V-dependent haloperoxidases involved in wood degradation: Even the simple molecular anion amavadin (Nawi and Reichel 1987; Rehder 1991), presumably just a kind of transport form for vanadium in fungus *Amanita muscaria*, exhibits a variety of versatile catalytic features in oxidative cleavage of aromatic and other C ring systems, phenol functionalization etc. Green plants in turn get better access to Mg, Mn (photosynthesis) and Ca for cell cycle regulation, etc. Zn is required by both plants and fungi but its complexes with bidentate low-$E_L(L)$-ligands are rather weak which is compensated by plants by delivering the (tridentate) citrate the Zn complex of which is somewhat more stable. In the same manner citrate additionally enhances uptake of Fe and Cu while no advantage is gained by citrate delivery for either V (required by many fungi) or Mg, and for Mn(II) the situation even is reverse: complexes of some bidentate ligands are more stable than the citratomanganese(II) species. Positions where the ligands are written correspond to their $E_L(L)$ values (cp. Table 2.2) while the abscissa gives the complex stability constants for bidentate ligands with the metal ions at the right side obtained from Table 2.2 and Eq. 2.4. Concentrations of "free" Fe(III) ions in a soil solution are exceedingly small (Scheffer et al. 1998; Sigg and Stumm 1994). For bidentate ligands with $E_L(L) \geq -0.12$ V, the vertical arrangement of the lines for divalent cations reproduces the Irving–Williams (Irving and Williams 1953) series: Ca < Mg ≈ Mn(II) << Cu(II) > Zn. Oxalate is produced by roots of many plants while 1,2-diphenols are prominent in humic matter (Scheffer et al. 1998)

general binding behaviour, that is, for ligands having $E_L(L) \approx 0$ V such as amino acids (cp. Figs. 2.1 and 2.9) while other values of electrochemical ligand parameters bring about the frequent (Irving and Williams 1953; Sigel and McCormack 1970) "permutations" among positions of certain element pairs in the series. As outlined before, $E_L(L) << 0$ V is frequent in biological material and both its soluble and polymeric components, hence here occur similar permutations also. When does this happen? The limiting condition is given by

$$-\log k_{diss} = x^* [E_L(L)] + c \qquad (2.4)$$

for an individual ion M

$$-\log k_{diss}(M) = x_{(M)}^* [E_L(L)] + c_{(M)} \qquad (2.4a)$$

for some second ion M′, accordingly

$$-\log k_{diss}(M') = x_{(M')}^* [E_L(L)] + c_{(M')} \qquad (2.4b)$$

Some "deviation" from the Irving–Williams series results if differences of ligand sensitivities get larger than those of intrinsic bond stabilities:

$$x_{(M')}^* [E_L(L)] - x_{(M)}^* [E_L(L)] < \{c_{(M')} - c_{(M)}\} \qquad (2.18)$$

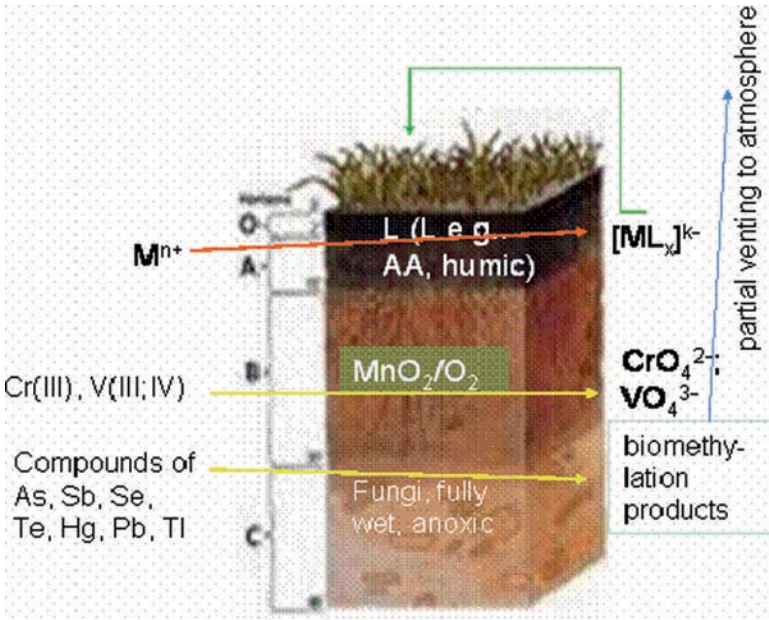


Fig. 2.10 Chemical processes which can alter speciation of various elements differ among different soil layers: various sediment layers can control passage of heteroelements into deeper soil or sediment strata by chemical reactions, with phenols, phosphate or As(V) retained in corresponding layers whereas other kinds of transformation either directly invoke biological activity (e.g. biomethylation), not to be mimicked by simple element-organic chemistry except for As (Cadet reaction) and possibly Co. MnO_2 is a potent redox catalyst capable of oxidizing Cr(III) to yield chromate(VI), Ce(III) to Ce(IV), phenols to catechols and muconic acids, etc. given there is O_2 while *Aspergillus* and other fungi can mediate biomethylation of As, Sb and many metals. If neutral species like $As(CH_3)_3$ arise by this process, they may diffuse out and escape into the atmosphere

or

$$(x_{(M')} - x_{(M)}) * [E_L(L)] < \{c_{(M')} - c_{(M)}\} \quad (2.19)$$

implying that the following value of electrochemical ligand parameter must be surpassed:

$$[E_L(L)] > \{c_{(M')} - c_{(M)}\} / \{x_{(M')} - x_{(M)}\} \quad (2.20)$$

When Zn is more enriched (larger BCF value, and $k' > 0$) in some biomass than Cu, Eq. 2.20 implies $E_L(L)_{eff} \leq -0.30$ V, with $BCF_{Mn(II)} > BCF_{Co(II)}$ in the same sample (*Daphnia magna*) a corresponding value of ≤–0.27 V is obtained (Fränzle and Markert 2000a, b). There are bioligands – phosphorylated species – which are capable to explain such extents of "anomalous" fractionation without invoking active transport; however, complex formation constants get rather low in either pair of divalent metal ions ($k \approx 10^3$). Fractionations of this kind thus would appear to imply rather high environmental concentrations of such ions, much into toxic levels as far as Cu or Co are concerned, but, in passing several transport steps and kinds of membranes, similar amplification of complex stability differences might take place – to be expressed in the k′ parameter – like observed in certain plants (e.g. *Ericaceae*). However, we consider aerobic organisms here while the +II oxidation states of both cobalt and manganese will not be maintained in oxidizing conditions at neutral or higher pH values when ligands like amino acids are present.

In any case, such values of electrochemical parameters for ligands relevant in biology almost from biopoiesis onwards allowed for broad and differentiated use of now essential elements much like in recent biomasses also >2.3 bio. years ago, without the necessity to have specialized transport systems at hand. Possibly, phosphate-containing regulating systems and transporters – besides the more advanced polyphenols, hydroxamates or proteins (chaperons) – involved in transportation of, e.g. Fe are relics of those days. Possible items of such transport include all of the more general essential elements except for Co and V. The edges to the central "window of essentiality" in the c/x

mapping suggest that changes of accessible oxidation states in redox-active elements did not thoroughly change the patterns or conditions of essentiality, especially concerning some transition metals close to these edges in their now familiar oxidation states (Mn, Mo, V, Co). Others close to or within this area after undergoing oxidation did never attain biocatalytic functions as far as can be assessed (Eu, Ce), while Mo and Ni were shifted to these edges.

Along a limnetic trophic chain, concentrations of many – including essential – metal ions tend to *decrease* rather than increase up the trophic chain (Fränzle and Markert 2002b); elements with highly negative ligand sensitivities x_{2d} such as Sr, Ba, La, Pr, Nd or Zr ($-9 \geq x_{2d} \geq -46$), but also Ni (x2d = +8.85), tend to be biomagnified by daphnia (two different species) eating phytoplankton followed by biodilution with the daphnia being consumed by fishes whereas there is no instance of the inverse set of biomagnification behaviors (Table 2.11):

For "switching" purposes (cell signalling, enzyme activation) no species can be used which is very abundant in the environment. An example is the role of Na in enzyme activation in terrestrial animals; this presumably would not work in seawater. Activation of metabolism by hormones containing iodinated tyrosines – once again typical of vertebrates – depends on the same condition even though the I abundance in OW is rather limited. There, however, are cases where there is biodilution in either stage of the trophic chain, notably with generally essential elements like Mn, Fe, Zn and I. Ce and Cr are depleted by fishes after eating zooplankton which latter would not alter the concentrations seen in its own (phytoplankton) food**.** Zn and Fe(II) which is the only water-soluble oxidation state, additionally enriched by photoreduction of Fe(III) in – even oxygen-saturated – freshwater (Fränzle et al. 2005) and thus is far more bioavailable than the higher oxidation state. Both Fe^{2+} and Fe(III) have positive x_{2d} values (Table 2.3) while $BCF_{alga/daphnia}$- and $BCF_{daphnia/fish}$-values for Cu or Al are regrettably absent from the above set of data. The oxidation state of Mn during biotransfer is uncertain.

Conceivably the number/volume density of metal ion binding site decreases when changing from daphnia to (planktivorous) fish while $E_L(L)_{eff}$ for biomagnification in fish should be close to 0 V. As discussed before, $E_L(L)_{eff} = -0.25$ V for biomagnifications of metals in *D.magna* which is far lower than that for both fishes and many kinds of aquatic plants including phytoplankton. Through the trophic chain, nitrogen is effectively retained in the consuming organisms, with Ni (urease; or Mn in animals), Mo (redox processes) and Mn involved in closing the internal N cycles while a larger part of C is oxidized up to CO_2. As a result, the C/N ratio familiar from ecological stoichiometry steadily decreases from phytoplankton via microcrustaceans up to fish (Fig. 2.11, from Fränzle et al. 2005):

It is necessary to better analyze the differences in $E_L(L)$ between daphnia and "typical" limnetic phytoplankton. The corresponding value for aquatic plants is distinctly higher than for *D.magna* yet it is negative, and bidentate ligands are involved as evidenced by the distributions of the above, mainly essential, metal ions. Hence metals with negative ligand sensitivity are enriched by phytoplankton whereas Fe or Zn undergo depletion with respect to their algal concentrations. The situation resembles that in uptake of metal ions from the rhizosphere by green plants, fungi or local bacteria.

Table 2.11 Biomagnification of elements in simplified trophic chains in freshwater (Fränzle and Markert 2002)

Symbol	Behavior (algae/ *Daphnia* spp./ fishes)	Corresponding elements	Commentary
MM	Magnification/magnification	None	Are metal ions not sufficiently lipophilic or ligand-affinic for ongoing enrichment?
MD	Magnification/depletion	*Sr*, *Ba*, La, Pr, Nd, Zr, **Ni**	
DM	Depletion/magnification	None	
DD	Depletion/depletion	**I, Mn, Fe, Zn**	Only essential ones
MN	Magnification/no effect	**Na**, Rb	Almost no coordination of these ions
DN	Depletion/no effect	Cs, Ag, **Mo**	Heterogeneous group; soft ions
ND	No effect/depletion	Ce, **Cr** (**Se**?)	Redox: cations/oxoanions
NM	No effect/magnification	Nb	Oxophilic
NN	No effect/no effect	None	

Bold letters: essential elements

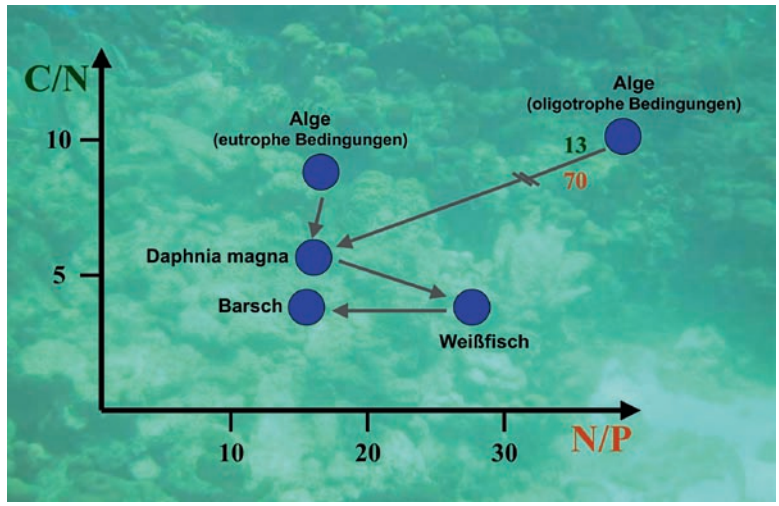


Fig. 2.11 Stoichiometric ratios (C/N vs N/P) in a limnetic trophic chain of four members (alga, water flea, planktivorous white fish, perch). The composition of algae (unlike animals, higher plants or bacteria) does thoroughly depend on nutrient contents of the lake and intensity of incident light (Loladze et al. 2000). Arrows point at the corresponding consumers in the trophic chain (from Fränzle et al. 2005)

Table 2.12 C/N ratios of different organisms vs terminal electron acceptor, ambient redox potential: the lower the redox potential of the terminal electron acceptor, the larger C/N will become

Kind of organism	Terminal e-acceptor (oxidant)	C/N (stoichiometric ratio)	Log (C/N)	$\varepsilon_{pH} = 7$ (minimal ε value where terminal oxidant can still be detected in neutral soil) [V]
Animal	Dioxygen	6	0.78	+0.55
Fungus (aerobic)	Dioxygen	7[a]	0.85	0.55
	Nitrate	?	?	0.33
Heterotrophic iron bacterium[b]	Fe(III)	22[b]	1.34	0.15
	Sulfate	120	2.08	−0.05
Clostridium	H_3O^+	About 140	2.15	−0.18
	Organic carbonyl compounds			

[a] Also holds for facultatively anaerobic fungi like baker's yeast; there, C/N = 8.1. This value (Fränzle and Moritz 2008, unpublished) corresponds to sporophore, for mycelia; C/N = 10–15 (see below)

[b] *Leptothrix ochracea*, whereas chemolithoautotrophic iron bacteria reduce CO_2 rather than oxidize ambient organic material by means of Fe(III), yet assuming the same C/N ratio

As we deal now with an ecosystem rather than with a single species organizing some extraction equilibrium with an abiotic environment, we can extend the above statements to ecological ones. This argument is uederlined by the following consideration from ecological stoichiometry: there inevitably is some N (and P) assigned to nucleic acids (and NTP energy reservoirs) in living beings, the more the higher the rates of cell budding are (Sterner and Elser 2002). So, with an efficient pathway of anabolic metabolism tapping whatever resource and energy sources (including photoautotrophy), more N and P will be allocated to these tasks in a healthy organism; conversely, with poor ways of delivering energy (e.g., heterotrophy based on weak oxidants like sulfate) from nutrients less metabolic activity is related to energy turnover and reproduction, thus C/N (and C/P) will increase the weaker the terminal electron acceptors are. Empirically there still today is a relationship between C/N ratios of ecological stoichiometry and the kind of oxidant which is used by organisms, described in the above Table 2.12:

From this a regression equation can be derived which links log [C/N] to the minimum redox potential required to sustain the corresponding metabolic oxidation as the oxidant must still persist in soil or groundwater, namely:

$$\text{Log (C/N)} = -1.898 * E_{pH=7} + 1.827 \qquad (2.21)$$

This – as yet unreported – relationship Eq. 2.21 is difficult to account for causally right now but possibly it is due to a larger extent of complete oxidation of consumed (devoured) organics into CO_2 which can be effected by stronger oxidants (oxygen, nitrate). In turn relatively more nitrogen is left behind in the consumer organism (although the latter will yet give away some N, e.g. as urea, as a rule). As noted before, C/N and C/S ratios also will have implications for the tendency of biomass to absorb, retain and fractionate metal ions in it: Whereas C/N becomes extremely large in such creatures dwelling at very low redox potentials, certain elements tend to be used exceptionally often and for multiple purposes in those organisms, e.g. W and Ni in clostridia and archaea, Ni also in plant urease and some plant species doing hyperaccumulation, whereas V piles up in (and partly is also used by oxic-living) ascidians, holothurians, fungi and by green plants producing linolenic acid (Emsley 2001). Possibly the precursors of the different groups of eucaryota did adapt to different milieus already during or even before the Precambrian oxygen disaster, concerning the recent data which place the "assembly" of eucaryota well back into anoxic times (some 2.7 bio. years ago). Even now, all four groups contain members which can sustain life without oxygen for quite a period of time, even some vertebrates can, and plant photosynthesis is markedly reduced by ambient O_2.

2.2.8 Implications of Some Theorems from Stoichiometric Network Analysis (SNA) with Respect to Stability and Function Biochemical Systems

Now, life is about reproducing some very complicated chemical gear – including accumulation and appropriate speciation of all the elements which are essential to that species – in an autocatalytic manner. From this point of view, a green plant is a device which produces and employs carbon compounds to obtain more C and increase its throughput/fixation rates of C (and all other essential elements) by (usually) identical reproduction of first the corresponding reactants (rubisco, chlorophyll and all the other proteins involved) and then that of metastructures (cells, entire organisms). Growth and reproduction thus correspond to **autocatalysis**. Autocatalysis does not only mean reproduction of some entity within the chemical network from components which are taken up from outside (similar to biological reproduction) but can also bring about "exotic" (Pota and Stedman 1994) chemical behaviour and kinetics, such as pattern formation, including chemical wavefronts, time lags before switching to another reaction channel ("clock reactions"), oscillations and even chemical chaos. In clock reactions (e.g. the "iodine clock"), chemical wavefronts, and chemical oscillators, the autocatalytic species uses to be the solvated proton (Pota and Stedman 1994) which is liberated from the solvent by some oxidant. This is why these kinds of non-linear chemistry are observed in few protic solvents only (water, methanol, formic or nitric acids).

There are certain formal rules which must be obeyed to maintain autocatalytic features of biological systems, like any other ones: inflow of autocatalysts or their precursors will not do but their ways of use must likewise be considered. While living beings must reproduce sooner or later to keep present for geological periods of time, the novel problem arises which are the conditions to maintain some component of this multicomponent autocatalyst in operation.

Stoichiometric network analysis (SNA) affords some framework of theorems which describe behaviours and (meta-)stability conditions of autocatalytic (chemical or nuclear) reaction networks. Hence it is more straightforward to use these semi-quantitative arguments on numbers of functions of some autocatalytic component ("larger than") rather than trying to obtain and formally analyze global models of the entire metabolism of some organism (which was feasible for quite few microorganisms only so far). Though there is an advantage in using qualitative or semi-quantitative arguments, this does not extend into coordination chemistry of essential ions and the reasons of the latter ("the natural selection of chemical elements"; Williams and Frausto Da Silva 1996) which eventually allow and demand for quantitative analyses and statements. *Nota bene*, the proof of essentiality by deprivation experiments following Liebig's minimum principle is

tantamount to demonstration that corresponding autocatalytic cycles exist and involve certain chemical entities. SNA thereafter can be used to work out the autocatalytic features from a plethora of linear-throughput (metabolic) or "background" chemistry. Whereas in plants or animals or fungi the items of linear metabolic throughput usually correspond to the essential ones, i.e. the autocatalysts (which is a requisite of heterotrophy), this may be different in chemolithoautotrophic organisms: oxoanions of elements like U, Cr or As can be used as electron sinks besides or instead of Fe(III), Mn(IV), sulfate, etc. by these microbes without bringing about any biocatalytic function of U or Cr or As in the same turn. So there is no autocatalysis (i.e. enzyme component role) with these elements but simply stoichiometric turnover which is "disregarded"/put to another category by the SNA approach. The "rule of three functions" is borne out from these considerations.

Since they are unlikely to be either accumulated to serve as some biocatalyst or else rejected for toxicity, elements (metals mainly) which are not essential but slightly toxic at most are best suited to analyse and understand binding and transport of element ions in organisms. Both properties/features and preconditions of persisting autocatalysis are

1. Some steady increase of turnover rates, that is, of total reaction rates, and
2. The availability of some resource (set) which can be exploited by the organism from which it derives the essential trace elements (besides others)

With the resource plentifully available, the corresponding species is likely to spread unless limited by other nutrient resources or by competition (Section 2.3.2) or else the essential trace elements get toxic if supplied in too large or unbalanced amounts (recall the antagonisms among essential elements (Fig. 1.2) also). Both a single organism and the total inventory of biomass of its species are going to lose some part of these essential trace elements anyway, be it by death of the organism, loss of certain parts (e.g. of leaves), with animals by excretion or – which is highly important on any trophic level – by being eaten within some trophic network.

Even though population oscillations – which can take place in either a regular (Lotka 1910) or chaotic (May 1976) manner – may still occur, there will be some kind of labile equilibrium among biochemical autocatalysis, increased use of resources and the various kinds of loss processes (with aquatic animals capable of using dissolved ions and metals bound to food parallely while the length of limnetic trophic chains apparently is limited by steadily decreasing concentrations of the said essential trace elements rather than the often assumed steady enrichment (Fränzle and Markert 2002) which is so frequently observed with unpolar organics). *Nota bene*, the rapid, "explosive" spreading of some species on whatever trophic chain usually is interpreted by ecologists or wildlife biologists as a strong indicator that "regulation has failed" somehow regardless whether some producer or consumer is concerned. Often such events, e.g. plankton blooms, bring about anomalous matter flows, too. This regulation is closely related to the loss processes at least insofar as essential elements are involved; the extent of feedback of Mg, Zn, … by leaf litter to the soil was thoroughly investigated (Bardgett 2005).

The metaboloms of organisms – even that of rather simple, unicellular ones – form highly complicated networks of interconnected chemical reactions, causing a problem of both description and analysis: whereas analytical data such as elementary analyses of biological samples (e.g. plant leaves) correspond to a certain point of time which may even circumscribe some stage of metabolic reorganization owing to changing environmental conditions. Here is periodically repeated sampling, including that of differently old leaves/needles/twigs, the only way to determine kinetics of possible changes, thus dynamics is not directly accessible. For example, if one uses yeast samples produced and stored in aerobic conditions and has them growing and reproducing in an anaerobic milieu (production of cider from yeast, apples and added sugar), Zn-containing enzymes become more important while Mo-based reductases are of no use to the yeast cells any longer. Yet it takes about 8 days (several generations of yeast cells) before the composition of *Saccharomyces cerevisiae* changes accordingly, steadily exchanging metal ions with the degrading apples and the liquid phase: more Zn, less Mo in yeast (Fränzle and Moritz 2008, unpublished work). However, dynamics are most important with living beings being throughflow systems reproducing catalytically (enzymatically) active materials in an autocatalytic fashion while other reactions are unrelated to growth and reproduction – but including detoxification or excretion of non-essential cat- or anions. Hence it is necessary to use some formalism which distinguishes such "colloquial", linear chemical transfor-

mations from the autocatalytic ones related to reproduction in the wider sense, including budding of cells in order to get beyond single-point-of-time representations. One formalism which isolates autocatalytic "core" processes – whether these be chemical or of some other kind – from others in a larger network is **Stoichiometric Network Analysis (SNA**), which was developed by Canadian chemist Bruce Clarke during the 1970s. Theorems derived from SNA (collected, e.g. in Clarke 1975, 1980) partly directly apply to description of biological systems from scales of molecules (biochemistry and bioinorganic chemistry) up to entire biogeochemical cycles and biocoenoses. For avoiding to go into complete modeling of some metabolom using SNA – as was done for *E.coli* (Schilling and Palsson 1998) and *C.elegans* before – one can work with semi-quantitative arguments focused on presence and "roles" of certain active components; the number of the latter is fairly small (≤30 essential elements and at most several dozen essential cofactors (amino acids, unsaturated fatty acids, vitamins) in a given species). Thus complexity becomes tractable, even recognizing additional implications already discussed (chapter 2.1) with regards to Gibbs's phase rule. SNA is about classification of different kinds of autocatalytic systems, discriminating them against non-autocatalytic ones in this very moment. As noted before, this method of systems analysis is by no means restricted to identifying features of chemical autocatalysis.

Corresponding behavior also is observed in man-made nuclear reactions (nuclear fission reactors or –bombs (Clarke 1980)), astrophysical processes (pp-II and pp-III fusion chains in main-sequence stars, with ^{4}He being the autocatalyst[5] species (lecture by S. Fränzle given at Zittau 2003, unpublished)), and dynamics of macroeconomics and technological innovation which unleash long waves and technology wavefronts with all typical dynamic and spatial features of autocatalysis (Fränzle and Grossmann 1998). Issues analysed by SNA which are more related to biology include the epidemic spreading of pathogenic organisms including viruses (Clarke 1995).

[5]Proton–proton fusion chain reactions (Bethe and Critchfield 1938): the first long-lived intermediate of nuclear fusion in low-mass stars, ^{3}He, adds to one ^{4}He to yield (radioactive) ^{7}Be which eventually produces **two** ^{4}He by capture of another proton and radioactive decay of ^{8}B (pp-III chain) or *vice versa* along the ^{7}Be decay product ^{7}Li + p (pp-II).

Autocatalysis is a distinctive phenomenon: while in "ordinary" catalysis the catalyst re-appears from the reaction apparently untouched, additional amounts of catalyst are actively produced in an autocatalytic cycle. As atoms are not interconverted during chemical reactions, this requires (all) the (elementary or otherwise essential) components of autocatalysts to be **extracted from** some **external reservoir**. After all this matter was extracted, some share of it is not introduced in and released as a product but rather retained, thereafter supporting and speeding up the reaction(s) steadily as amounts and possibly also concentrations of autocatalysts increase. At first glance, such a system may appear doomed to undergo runaway dynamics ("explosion"), but, apart from the limited speeds and rates of autocatalyst resupply from the environment there are also other mechanisms which usually limit kinetics even though non-linear behavior (bistability, oscillations) may not be precluded:

(a) Side reactions consuming some autocatalyst or precursor thereof counter-act autocatalysis
(b) Often, in particular in biological systems, autocatalysts cannot operate "on their own", as semi-isolated entities but must form cooperative structures to get activated at all

As was mentioned before, reproduction and cell-budding – so typical for living creatures – can be taken to represent autocatalysis. As autocatalysis includes the *chance* (see below) for exponential growth in certain cases, biological or biochemical transformations will prevail over non-autocatalytic similar or identical ones in the long term (autocatalysis does not produce exponential growth kinetics in every case (Severin et al. 1998) but rather often the dynamics of autocatalyst concentration (and turnover) increase is restricted to logistic growth, as with many peptide replicators). Even though, the redox processes producing or consuming Fe oxides, taking away or releasing Fe^{2+} induced by iron bacteria (*Leptothrix, Gallionella* or *Geobacter*, respectively) will out-compete spontaneous oxidation of aqueous Fe(II) or of olivine (peridotite), magnetite in air or reduction of ferric oxides in reducing ground or ambient water levels before long; as a result, these processes are mainly biogenic on Earth. In some cases, the non-biological, non-autocatalytic transformation does not occur on Earth at any appreciable rate, e.g. with N_2 or sulfate reductions.

Obligately cooperative autocatalytic phenomena in biology cover quite a spectrum of scales, from formation of multienzyme complexes by sexual reproduction up to endo- or exosymbioses like with lichens. Population dynamics then rather suggests that autocatalysis, given the "obstructive" effects cited above, is hardly sufficient to maintain it, bringing about linear chemical kinetics, survival and a modest reproduction rate unless, of course, novel resources are opened by either migration, local chemical changes (erosion, anthropogenic input) or/and evolution (e.g., bacteria "learning" how to use antibiotics or xenobiotics as nutrient or C sources). As will be shown later on, supply and composition criteria in lichens must be that precisely balanced, that is, by using Eq. 2.4 it is possible to calculate which kind(s) of ligands can only used in an alga-fungus-system to extract essential metal ions from the environment and meet demands of either organism (alga or fungus): $E_L(L) \approx -0.18$ V is best matched by oxalate and less so by 1,2-diphenolates, and oxalate is employed in metal retrieval by most lichens indeed (Purvis 1984), up to the extent that the "etched" or "leached" support minerals under lichens become covered by secondary oxalate phases such as whewellite or moolooite, oxalates of Ca and Cu(II), respectively.

However, it is crucial to understand that such cooperation is not a blueprint for success in and among autocatalytic systems but rather brings about hazards up to "hard" (exterminating one or several competitors, often all but one) selection dynamics (Eigen 1971). Clarke (1980) gave proof that this way of negative feedback in cooperating autocatalytic feedback loop systems, paradoxical as it may appear, is not a feature of certain chemical systems like nucleic acids or restricted to peculiar chemical kinetics/certain kinds of reaction but on the contrary must occur in any autocatalytic metasystem constructed from cooperating autocatalytic subunits, given autocatalysis displays exponential kinetics in the non-obstructed system. Yet results taken from the Biological System of Elements can be used to prove that neither symbiosis nor sexual reproduction are "paradoxical" features, which would be hard to reconcile with their commonness in living nature. SNA likewise applies to different hierarchical levels of biology, encompassing biochemistry, physiology and ecology alike (Fränzle 2000 in Breckling and Müller (ed.)), with this work being focused on the biochemical or molecular level. On this level, we especially consider the array of biocatalytically active metal ions and their specific chemical features, e.g. organometal transformations at Co, hydroxylating reactions at Fe or Cu or catalyzed stepwise hydrolyses with Mg, Zn or sometimes Co centers again. So let us look upon a general, formally autocatalytic system.

There, an autocatalyst A is reproduced by uptake of its material precursors from the environment, the latter being released/extracted from some resource (soil, "food" and so on) to which it was bound as some substrate adduct A-S, making more A besides some product P. The most simple case of this transformation is

$$A + A\text{–}S \rightarrow 2\,A + P \qquad (2.22)$$

where P also may be some mixture of compounds, which in fact rather is the common case. Likewise, A can exist in multiple different binding forms (e.g. isoenzymes with identical catalytic centers but different sugar attachments or responses to some chemical changes) A, A′, A″ and so on, catalyze several different chemical reactions (Vallee and Williams 1968), or even multiply during autocatalysis more than just doubling by substrate (resources) transformation:

$$A + A' + A'' + 3\,A\text{–}S \rightarrow 2\,A + A' + A'' + 3\,P \qquad (2.23a)$$

However, our interest is focused with the set of (possibly essential) chemical elements which can be absorbed in different speciation forms (e.g. N as NH_4^+, nitrate, urea or even unchanged glycine (Bardgett 2005)), neglecting these very speciation forms, which simplifies Eq. 2.22a to yield

$$3\,A + 3\,A\text{–}S \rightarrow 6\,A + 3\,P \qquad (2.23b)$$

which corresponds to sixth-order (!) autocatalysis. **Efficiency** of autocatalysis is given by its autocatalytic order O_{AC}, which denotes the number of autocatalyst entities formed after one reproduction step,

$$O_{ac} = R_{cat} \qquad (2.24)$$

e.g. $O_{AC} = 4$ in the acid-catalyzed chemical wavefront reaction

$$I_2 + SO_2 + 6H_2O \rightarrow 2I^- + SO_4^{2-} + 4H_3O^+ \qquad (2.25)$$

Thus four protons are produced when going through the cycle once. In chemical oscillators usually the proton is the autocatalyst species, too (that is why oscillators as a rule do operate in protic solvents (water, methanol, formic acid) only), with $O_{ac} \geq 5$ in several cases (Pota and Stedman 1994). From this value, stoichiometries of such processes must be substracted which will decompose, bind or remove (from the reaction network) the autocatalyst species. Non-linear modes of chemical

behavior are most frequently observed in open systems which are part of their classification (Eiswirth et al. 1991b) whilst oscillations are seen in multienzyme complexes in vitro also. On how the general balancing is done, cp. Chapter 2.2.7; Section 2.2.11. As for the efficiency or yield of autocatalyst (assembly) replication, the common value is 2 in unicellular organisms which reproduce by budding while in higher plants it depends on the specific mode of reproduction.

2.2.8.1 Additional Criteria from Stoichiometric Network Analysis (SNA)

SNA can reveal the features/processes which bring about autocatalysis (AC) in a general reaction network which processes matter. It does not matter whether the said autocatalytical processes depend on metal ions and thus are linked to complex formation constants; AC is also effected by protons (acid production during oxidation), whether there are any other redox or radical chain processes or we deal with chemical transformations at all (Clarke 1980). There also may be AC in nuclear processes, e.g. stellar nucleosynthesis (with ^{4}He as AC agent). Thus, SNA is "blind" towards reasons (particular biochemical functions) or details other than those borne out from network topology (Eiswirth et al. 1991a,b). A full-scale description can be achieved also for such cases where essentiality was established (or is highly probable at least) yet there is no idea on the biochemical functions now, e.g. with essentialities of V, As, Cd, B or Si (for different species/taxa or different levels of uncertainty). What really matters in dynamics and stability of autocatalytic systems is the **number** of functions which can be clearly attributed to one single element, which need not be catalytic but shall all contribute – as will be shown below – to construct some stable state – or fail to do so.

Critical cycles tend to be more sensitive towards processes which obviate, quench or destroy some part of the corresponding autocatalyst than strong ones. Collapse into a weak cycle thus is more likely, and thus can be brought about by smaller changes or perturbations, e.g. by eating, than with strong cycles. As a result, consumers might **constrain** the array (manifold) of essential elements in plants or bacterial producers (chemolithoautotrophs or phototrophic bacteria such as thiorhodacees) which they devour. In fact, the average number of different essential elements is **smaller** than with animals though autotrophy should entail more difficult and diverse chemical transformations to be accomplished by some organism. Nevertheless especially higher plants can do without Na, Sn, As, F, I, V, Cr and Co which many or all animals require, while there is but a couple of established or presumed functions for the three transition metals mentioned last and, in addition (or as a result), the required amounts of these metals are tiny (a few μg/day in man, for example). Herbivorous or bacterivorous animals of whatever size thus apparently control what producers might base the catalytic features of their metabolisms on – excluding trace metals like V, Cr or Co, but not Ni (urease!) (this author once came across a population of amphibian tadpoles which mainly fed on colonies of iron bacteria thriving around ferrous springs in a (now destroyed) larger pond in the Dolomite Mountains (Italy), and man-fishes *Proteus anguineus* likewise feed on cave loam which often contains appreciable amounts of autotrophic bacteria). Yet, they will not keep them from (also) accumulating (some of the) elements they cannot make use of, and hence primary consumers in turn are capable to obtain them from their food, especially as demands of such elements tend to be very small. This latter once again is due exactly to the rather limited number of functions accomplished by such (ultra-)trace elements, bringing about critical rather than strong AC cycles and rather low AC orders. Of course, animals often are in a position to obtain the above elements from all food, soil, mineral or water (terrestrial or freshwater) sources directly while the mentioned small required amounts presumably abolish the problem of keeping their budgets inside the body even if environmental concentrations are very small (like in ocean water).

From earlier parts of Chapters 1 and 2 it became obvious that stoichiometric network analysis favourably combines with information from coordination (bioinorganic) chemistry to provide an account of essentiality patterns. Yet it does not suffice that some chemical element can or might be useful for catalyzing transformations in a chemical environment akin to biochemistry, but for fulfilling any biological function it must be retrieved from either environment or (in heterotrophic organisms) from food on a regular basis in sufficient amounts to become integrated into some autocatalytic cycle: as an essential element will remain so also in forthcoming (division product) cells and generations, it must be made available from environment or food (generally speaking, from some reservoir) steadily to accomplish reproduction, conservation of

matter necessitating external uptake. Thus bio(-inorganic) chemistry demands for an almost continuous inflow of at least 13 different chemical elements.

Generally speaking, in both plants and other organisms autocatalysis occurs on quite different levels of **hierarchy**. In ecosystems such as forests there are also interactions among different plants, (organized by) mykorrhiza, and other fungi, transporting metals (and nitrate, various organics) from one to another or competing for mineral resources. If there is precipitation of metals such as Mn, the topology of the network (sensu Eiswirth et al. 1991 a, b) may change also, inverting its response to yet other, secondary perturbations. It should be noted that all data taken on plants, etc. from the native environment (Markert 1996; Fränzle and Schimming 2008, etc.) are by themselves influenced by such interactions, although the differences observed among co-existing plant species demonstrate that "smearing" is rather limited. Of course, even if effects were more pronounced, the more fundamental criteria such as the three-functions-rule are not touched or altered, and the confinement of the essential elements to but a small region in the c/x map (Figs. 3.1 and 3.2) is not levitated either, the less so as apparently grassers are capable of limiting the numbers (and indirectly the ranges of chemical properties) of essential elements in producers, including green plants. The only parameter which changes indeed will be efficiency of supply/input (resorption) and geobiochemistry of certain sinks, that is, exit **rate** rather than exit order.

2.2.9 Matter (Flow) Balance, Metabolic Strategy[6] and Estimation of Loss Processes (Exit Order) Within Autocatalytic Biochemical Cycles

Often biological or biochemical functions are not realized over all the time throughout the lifecycle of some organism but rather operate for limited times and occasions such as reproduction, metamorphosis (role of iodine in amphibia), synthesis of hormons or other signaling components or of anti-eating poisons or repellants or detoxification agents like metallothioneins. In such cases, the autocatalytic order related to these compounds will be lower than for continuous use; this phenomenon is rarer in plants than in animals while the three-functions-rule does apply in an unchanged way, of course. Yet, given the oscillation and autonomous ways of excitation in strong cycles, situations can occur where such temporally limited decreases of AC order will increase stability; in addition, a system which can be (meta-)stable over many generations must obey other criteria like Gibbs's phase rule to escape stability problems which arise from a large number of organs.

According to Eq. 2.24, the AC order agrees with that of the functions; $O_{ac} = R_{cat}$, which produces a "broken AC order" at first glance for the case now discussed. However, the effect which gives rise to the three-functions-rule becomes operative only over a larger number of cell divisions or even generations. Thus, the part-time reduced O_{ac} will not cause the element to vanish from the essentiality list, but there will yet be a change of stability in the reaction network. Unless there were side-reactions which consume some part of A, some of it getting excreted or lost otherwise, e.g. becoming precipitated or exhaled in hardly soluble or (fairly) inert, thus (for most organisms) no longer bioavailable forms (Fe as vivianite $FePO_4$, N as N_2, etc.), reactions of kinds (2.22) would bring about a doubling of turnover rates in every single cycle. As far as biological systems are concerned, there is a particular event limiting the spatiotemporal effects of autocatalysis: death of the corresponding organism. In addition, the specific catalytic entities are difficult to hand over as such to the next generation in metazoans (except for certain plants growing from separated parts of their mother plant and enzyme transfers via milk in mammals) and are not indefinitely stable, with some part of the catalytic heteroelement of metalloproteins or selenoproteins being excreted rather than re-used after degradation of an enzyme molecule. Elements like Mo, Ni or V which rather form critical cycles nevertheless are prone to more grave changes than those dominant biometals such as Fe, Mg or Zn, let alone the principal non-metals C, H, N, O, S, P. After all, an exact count of the number of functions for any chemical element in an organism would imply the complete knowledge of all the metabolic pathways and involved (possibly metallo-)enzymes (as was achieved for *E. coli* by Schilling and Palsson (1998)), a set of data which is not either available from "mere" genetic sequencing like it was done for *Arabidopsis thaliana*

[6] "Strategy" here corresponds to some "evolutionary stable strategy" (ESS) as derived from biological applications of game theory (Szathmary, Maynard Smith).

as the first species of higher plants. Genetic sequencing just reveals the possible sequences of all possibly expressed proteins without giving information on, e.g. the extent up to which one element – e.g. in a metalloprotein – can be replaced by another, say Zn by Co or Mg by Mn, which reduces O_{ac} of the former element even though enzyme function is maintained: what is addressed and identified by genetic sequencing is the plain protein matrix, the apoprotein, which is identical and remains untouched by this replacement. It is impossible to deduce a clear-cut relationship between the sequence derived from the DNA sequence and the likelihood that some certain metal ion be introduced into the apoprotein at the then active site.

Considering the principal non-metals of biochemistry now, C and N are most directly involved in autocatalytic sequestration of metals in a cooperative manner: proteins – which invariably contain carbon – are also involved in CO_2 assimilation (rubisco) while nitrate reductase or transaminases which promote reduction and "organification" of N generally contain nitrogen also. In terms of SNA this means that the exit step of some autocatalytic cycle and the autocatalytic process in another (in the same cell or at least same organism) are not only chemically coupled to each other but rather **identical** with respect to both the process and thus also their (interconnected) reaction orders. In autotrophic organisms, internal integration of metabolic processes is most pronounced, implying the ability to accomplish reductions of all C, N and S oxospecies. As a result, many of the element (here: non-metal-)based cycles will become just critical or marginally strong in plants or autotrophic bacteria even though a huge number of compounds contain (each of) these elements. However, in animals these reductions are of far lesser importance; for those iron bacteria which use Fe(III) to oxidize organic compounds and obtain metabolic energy then used to synthesize bioorganics from scratch (CO_2, SO_4^{2-}, various N compounds) rather than just doing heterotrophic metabolism ("iron breathing"), the situation will be different as they require the complete apparatus of autotrophy and in addition must take up very much Fe but there is no detailed information yet concerning their essentiality patterns. Aerobic Fe oxidizers like *Leptothrix ochracea* contain appreciable amounts of S (C/S ≈ 20 (stoichiometric ratio), rather than the "usual" C/S ≈ 500), with sulfur donor sites possibly involved in Fe uptake and transport. Animals have both larger uptakes and losses of C but do not need to proceed it as thoroughly and diversely, and the same holds for N or S. (Topological) connectivity of metabolic cycles being lower, there is more potential for strong autocatalysis related to quite different and diverse autocatalyst species, and thus animals (and other heterotrophs such as fungi, many bacteria) might "try" and successfully use elements like V, Cr or Co while they keep plants from doing so by extensive grassing.

Concerning selenium, there is an interesting difference between S and Se, the latter prominent in thermophilic clostridia: in spite of the chemical similarity between S and Se no protein is known yet – whether it is an enzyme or fulfils other functions – which sports more than one atom of Se and thus but a single selenocysteine residue (Kyriakopoulos et al. 2004). Se is used in difficult-to-achieve hydrogenations, e.g. those of CO_2, or of xanthins (Dürre and Andreesen 1989), likewise in similarly difficult iodine cleavage in iodothyronine or C–N bond cleavage in glycine reductase (Arkowitz and Abeles 1990) which affords acetyl phosphate (besides S–S cleavage in glutathione peroxidase) but enzymes which reduce selenium precursors like SeO_3^{2-} as a rule do not contain Se (selenocysteine) themselves. So there is **no** autocatalysis concerning selenium but only crosswise catalysis concerning Se in animals or clostridia on the molecular (enzyme) level but of course there is (presumably strong) AC in the cell level. These biological systems are thus constrained with respect to hierarchy levels and "organization" of autocatalysis.

Strange enough, there is no specific coding in DNA translation for selenocystein, the only proteinogenic amino acid which contains any other element than the five principal non-metals besides P. With use of Se separated from "classical", molecule-level autocatalysis (Se (selenocysteine) uncoupled from Se sequestering or reduction in/before selenocysteine biosynthesis), it becomes the more noteworthy that there is no specific codon for this amino acid (Leinfelder et al. 1987; Engelberg-Kulka and Schoulaker-Schwarz 1988); apparently under these circumstances strong cycles are organized in another way (the argument cannot be extended as there are no other elements with similar uncoupling of AC hierarchy levels which made their way into proteinogenic amino acids (chemically speaking, e.g. As or easily biomethylated metals like Co or Hg could also behave like this).

Of course, in autocatalysis of their own fixation C, N, S "suffer" most from oligomerization of autocatalyst speciation forms, much more than, e.g. Mo or Ni as an enzyme molecule contains literally thousands of C atoms and some 100–300 N atoms for just a single small peptide chain. Carboanhydrases in which Zn may be replaced by Co or Cd (Price and Morel 1990), one of which is definitely Cd-dependent (Strasdeit 2001), occur in these algae generally as a multitude of isoenzyms, bringing about some "redundancy". As they all catalyze the same reaction, the contribution of any single such enzyme to fulfillment of the three-functions-rule is <1. If Fe and V replace each other in fungal oxidoreductases which process identical kinds of substrates or when Cu is replaced by various metals in arene hydroxylases, etc., the result will be similar: for "plain" substitution, the contribution will be zero concerning either metal biocatalyst whereas isoenzymes cause $0 < \Delta F < 1$, where ΔF is the contribution of a given isoenzyme (keeping the metal center constant if there is any) to the three-functions-rule; 10 or 20 isoenzymes jointly catalyzing three reactions are not at all "better" than just three quite different metalloproteins all containing, say, Mn (in SNA terms: the "roles" of autocatalysts may be distributed among different AC speciation forms without changing reaction network topology or dynamic features such as stability in given conditions (reactant concentrations, flow rates, temperature, …)).

Possibly also other non-metals, e.g. arsenic in marine organisms where As may be involved in biosyntheses of unsaturated lipids (Irgolic 1986) using arsonium-moiety-containing arsenosugars or arsenolipids in much the way of P(phosphonate or phosphinate)-based Wittig-Horner reagents. This cycle apparently also requires zinc, besides As (Leonard 2002). Notwithstanding the exact mechanism and the multitude and biodiversity of marine organisms which accumulate and obviously use (as kind of biocatalyst(s)) arsenic, there once again is no autocatalysis here: arsenate(V) reductase, e.g. from *Staphylococcus aureus*, does not contain As itself, and As biomethylation is achieved by As-free sulfonium salts (S-adenosylmethionine) and corresponding enzymes.

Concerning **spatial** effects inside a higher plant, preferential grassing of above-ground parts of terrestrial plants, in particular leaves and fruits, by animals will alter the elemental composition of what is left over from the plant after being partially devoured (subterraneous insect larvae feeding on roots will exert an opposite effect, that is, preferential extraction of elements bound to be retained in the roots which also means that these elements are unlikely to be essential). Aboveground grazers are thus more likely to limit the range of elements which can be essential to a plant (there is no such distinction in aquatic plants). For oligofunctional elements like Mo, Ni or B there apparently is no dramatic effect in terms of the three-functions-rule. SNA permits to describe matter transport up to ecosystems level including processes in trophic chains just because this approach is independent of hierarchical levels in some system. For avoiding toxic effects, e.g. by formation of reactive oxygen species, some substantial part of metals such as Fe or Cu must be stored in particular forms rather than directly "shuttled" to metalloproteins or freely floating around as an aquaion (cp. Rosenzweig 2001; Tottey et al. 2005). With cell budding which represents a case of "successful" autocatalysis (disregarding tumor formation which usually is accompanied by considerable modifications and "simplifications" of metabolic pathways rather than just "opting out" of control of cell division processes), turnover of organic substrates and resource metals doubles indeed. The sophisticated representation in Eq. 2.23a takes account of the fact that an autocatalyst often is represented by different isoenzymes, or even by enzymes dedicated to quite different transformations, with substrates of one enzyme not reacting with another. The complete set of such reactions promoted by the same heteroelement in biology (or at least in one given species of living beings) corresponds to the set of functions of this heteroelement, say those of Zn in dandelion or some other, nonliving yet reproducing autocatalytic system (e.g. some virus for which living cells are the reservoirs). Now return to SNA: one important term is the **autocatalytic reaction order** which is just the number of autocatalysts differing in both chemical structure and catalytic function but relying upon the same element and joined in the same system, e.g. an organism. For example, when in some species there are 20 enzymes all containing (e.g.) cobalt (in whatever binding form, including cobalamine) which catalyze 20 different transformations, the AC order of cobalt will be 20 if there are no losses whatsoever. In the real case of non-zero losses via different channels including demise of some members of the species the AC order will be lower, with every single loss process to be subtracted from 20.

How large an AC order, how many biocatalytic functions associated with a certain element, will be required to sustain autocatalysis altogether? The various loss processes and their numerical contributions will be considered in due course below. Unlike the approach used by Eiswirth et al. (1991a, b) who focussed on topologies of autocatalytic feedback loops to understand the dynamics of chemical oscillators, we are more concerned with numeric aspects of stability, the more as oscillations are rare in biochemistry. The capability of an element to get and maintain biological functions which, due to the necessity to reproduce, is no longer just a matter of chemical properties but can be traced to them ("window of essentiality" for metal ions) but takes additional criteria. As a kind of "bonus" this allows to advance from our starting point of considering abundances of chemical elements in biological samples, then almost unrelated to their functions, to analyze the relationship between amount and functions, with some (at least most likely) essential elements being present in man at µg (some 10^{-7} mol) levels only, e.g. V, Mo.

On the top levels of ecosystems and biocoenoses, however, there is no autocatalysis anymore as these systems do not (cannot *by definition*) reproduce. Rather, they form sources or sinks for elements, acting as a kind of "environment" for elements autocatalytic to at least some members of the embedded biota. This includes cases where some essential elements becomes deposited (Fe by iron bacteria, Ca by corals, etc., S by sulfate reducers) or transferred to the atmosphere, thus vented in a more (Se, As methyls) or less reactive (N_2) form, thus increasing exit orders. With always but a part of the metabolized element mixture being locked up in biomass over longer periods of time, the situation is "asymmetric" on each trophic level.

For first-level autotrophs (producers), sufficient admission of substrates may become an issue even though assimilation rates of 40–100 mol C m^{-2} a^{-1} may be reached (Bardgett 2005), as an increase of CO_2 in the gas phase brings about increased (but less than proportional with p_{CO2}) growth rates of plants. Moreover, there may be limitation by almost any of the essential elements, thus the importance of using fertilizers. Some of the essential elements are liable to considerable geochemical or geobiochemical loss processes, forming insoluble phases outside the organisms, e.g. Mn (and to a lesser extent Fe) with all aerobic organisms (Sunda and Huntsman 1988) or Ba with desmid algae when the waters are rich in sulfate or P-eutrophic (Woelkerling and Gough 1976). In the latter case, biodiversity (the number of desmid species which locally exist) is inversely proportional to the concentration of such species capable of precipitating Ba which means that at least for Ba among the desmides, there is an exponential distribution of required amounts, possibly due to some changes in composition of the sequestering and transport species (the functions of Ba in desmides are entirely enigmatic).

Anyway, when there are corresponding precipitation equilibria with Fe, Mn, Ba or other essential elements, or the latter get converted into forms from which they cannot be retrieved at substantial rates otherwise (N_2; N_2O in aerobic conditions), the loss (exit) order will increase and/or the access will be decreased. In either case, the "net" autocatalytic order is decreased, although neither Fe nor Mn nor N were eliminated from the list of essential elements by this effect (there are some organisms (including certain insects, bacteria and fungi) which can do without Fe, most can without Ba, but none will survive lacking N or Mn), can still fulfill the rule of three functions. The number of biorelevant transformations which can be accomplished by a certain element will also depend on the external chemical conditions, including redox potential (for redox-active elements as well as those which react with sulfide or organic groups in some way), temperature, pH (in acidic conditions, more cations of oxophilic elements (VO^{2+}) or also onium ions may be transported through membranes while weak acids may pass through lipid membranes as neutrals now, some of them [HCN, $As(OH)_3$] then causing grave problems). So, when there are additional possible functions in such exceptional conditions, "uncommon" elements may become essential (W in clostridia and – especially hyperthermophilic – archaea). Other organisms – like iron bacteria, e.g. *Leptothrix ochracea* (Fig. 2.12) – provide oxide-hydrate concretions of essential and other elements in a stoichiometric way, losing them through interfaces such as cell membranes, epithelia, stomata or the digestive tract, contributing to the exit order once again. Although there is no clear-cut reasoning for this assumption as yet, many people believe that hitherto multifunctional (metallo-)proteins underwent divergent evolution as to increase substrate specifities in the successors while changing but a few amino acids along their sequences (Yoshikuni et al. 2006). The rule of three functions shows why there is such a tendency: it does suffice to induce minor changes of ligand environments of some

Fig. 2.12 A colony of *Leptothrix ochracea* (some 15 cm across) in a small spring (producing 1–2 L water/min) among roots of a tree does locally control access to Fe (and probably Mn, Al, REEs) by this tree and nearby plants. Oxygen is depleted to about 0.3 mg/L by the bacteria from directly at spring outlet up to outflow into a creek (*front, below picture area*). Czech Republic; October 2008, photo taken by the author

metal ion to alter its capability to attract and convert certain substrates out of a collective which hitherto all underwent catalytic reactions to some extent, thus increasing selectivity and "effective" numbers of functions, which in turn stabilizes the role of the corresponding central ion in biochemistry.

The numeric argument concerning conditions of essentiality does apply to all the elements, some of which – like Fe, Zn or Mg – fulfill much more than three functions. So apparently the corresponding metal-based auto(bio-)catalytic cycles should be strong, and thus unstable, with possibilities for oscillations, bistability and so on. In vitro there is an example of oscillations in a Zn-based multienzyme system, namely, glycolysis. Thus one might assume the description of their biochemical behavior and its features to be much more demanding than for critical or "just strong" cycles put forward by, e.g. Mo, V, Co or the like. In reality, the task is less daunting for two reasons at least:

(a) The cycles are strongly coupled among each other (see Figs. 2.13 and 2.18, especially for magnesium in higher plants). Two completely coupled autocatalytic cycles in which one consumes the products of the other to "feed" the former with its own products, are by definition critical: the exit and autocatalytic orders must then all be identical.

(b) The individual "roles" within coupled AC cycles need not be attributed to or associated with one (chemical) species per each cycle but can be distributed among several ones, e.g. with isoenzymes. For example, in the lead chamber process it is futile to discuss whether NO, NO_2 or the nitrosyl cation NO^+ of the blue-violet "lead-chamber-salt" $NO(HSO_4)$ is the " real, genuine" catalyst: as all are equally involved in transfer of oxygen from O_2 to (aquated) SO_2 to produce half-concentrated sulfuric acid, the cycle can be started with any of these intermediates, all are involved and all are required to close the catalytic cycle. They form some **collective of intermediates** all contributing their parts; in addition, there may be different intermediates focusing convergently into the same catalytically formed product by different mechanisms or – more often – forming different by-products. Although product selectivity in bioinorganic chemistry is really admirable, there are by-products also, especially when the controlling chiral and membrane matrix is partly dismantled in in-vitro biochemistry.

The general feedback pattern does change in neither case (Eiswirth et al. 1991b; Fränzle and Markert 2003).

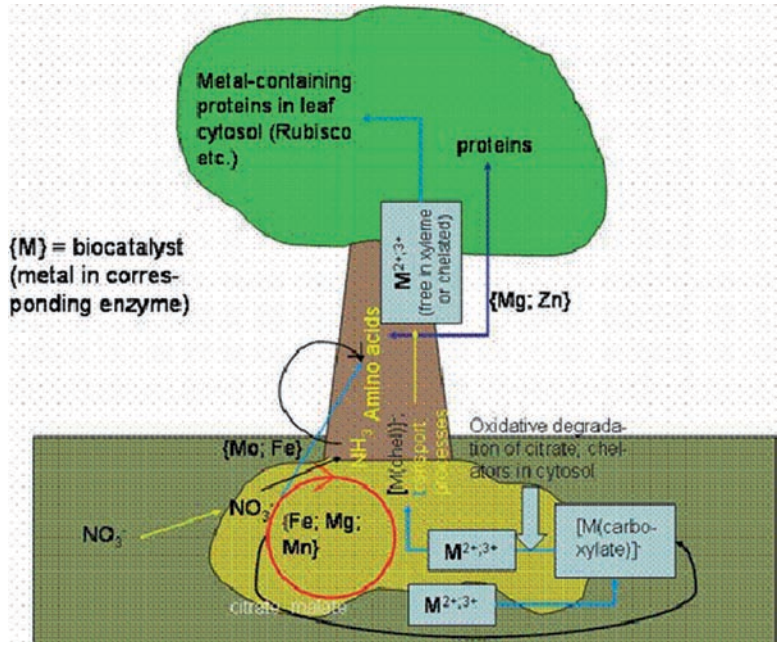


Fig. 2.13 A simplified sketch of the combination of uptake, transport and autocatalytic function in terrestrial plants. The rhizosphere is given in lighter grey to distinguish it from other soil regions. By their root system, terrestrial plants obtain water, metal ions dissolved therein, anions like nitrate (*lower left side of the picture*), sulfate or phosphate, etc. Chelating ligands like citrate, malate – both derived from the tricarboxylate cycle – are crucial in mobilizing metal ions. During further transport along upper roots, shoot and photosynthetic organs many of the metal ions change their speciation forms several times. Some part of them ends up in the biocatalytically active component of metalloproteins; these may be located in all leaves, root or shoot. The tricarboxylate cycle is marked; parts of the products are obviated into both metal mobilization and amino acid synthesis (over a couple of intermediates). Neither structures nor chemical classification of secondary chelators in the (*larger, upper*) roots or shoot are known in general (Clemens et al. 2002). Like commonplace in chemical notation, catalyst ions (metals which become active in metalloproteins) are denoted by swift brackets {M} disregarding issues like speciation or oxidation state (which may periodically change in a catalytic cycle anyway) whereas complexes are shown in square brackets. To yet allow for effective growth, the "investment" of organics to obtain metal ions from soil must be kept as small as possible

For the "negative" case, the impact of cytostatic agents into the cell cycle does occur in a way which can be described by the most simple, elementary (Eiswirth et al. 1991b) chemical feedback loops (Fränzle and Markert 2003). Both certain ecochemical effects and the impact of competing elements may bring about obstructive effects – concerning cell budding or reproduction, likewise – in milieus burdened by heavy metals or influenced by some kind of fluctuation. Kind and topology of biochemical feedback networks may be inferred from pieces of information concerning such obstructive effects (Eiswirth et al. 1991a; Fränzle and Markert 2003), for example, those of Cd instead of Zn, REEs instead of Ca or Pb blocking porphyrin biosynthesis by removing Zn from the relevant enzymes. Sinks are less important than sources here while on higher (heterotrophic) levels the situation will be reversed, often causing concentrations of metals to decrease from one trophic level to the next (Fränzle and Markert 2002b; Sterner and Elser 2002). Thus for plants the focus may be placed on uptake challenges and thus on coordination chemistry for the dominant metabolic cycles – that is why Mg was selected for discussing an example (Fig. 2.18).

Plants may even accomplish such loss of heavy metals without being devoured at all: heavy metals taken up before get bound to phytochelatins or metallochaperons and are then released through the plant surfaces or be abandoned by leaf littering. Many plants which do so contain highly toxic yet effective ligands in large amounts (in fact, these metabolites are toxic to man and many animals exactly because they are potent ligands), e.g. certain alkaloids in oleander, CN^- in laurel or (young leaves of) eucalyptus

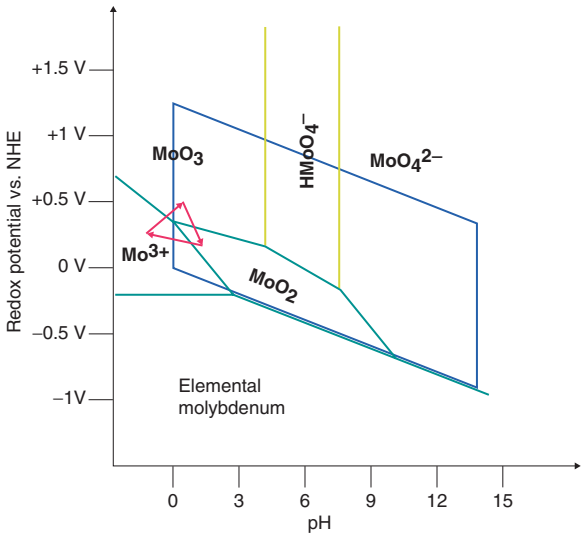


Fig. 2.14 Pourbaix diagram of molybdenum (redrawn after Coleman 2003). Rather than interacting with solid, aqueous MoO_3 and MoO_2, respectively, molybdoenzymes contain some (dithio-)molybdato pterin complex which keeps the metal suspended (*nota bene*, Mo is embedded inside a solid polymer matrix here, and in many animals and fungi the pterin is an essential cofactor which they cannot easily produce themselves nor independently link it as a ligand to inorganic [thio-]molybdates either but must intake the entire, linked Mo pterin system (Kaim and Schwederski 1993)). While the effective triple point Mo^{3+}/MoO_3/MoO_2 would be close to pH 1 and +0.3 V in "free" aqueous medium, the redox potential is increased by replacing hydroxo ligands with pterin to a level suited for nitrate/NO_2 turnovers, that is by >0.5 V. The vertical lines do not construct triple points among redox states as they simply correspond to protolytic equilibria. Besides nitrate reduction, other reactions can likewise be tailored to occur at pH ≈ 7 and intermediate potentials by this ligand exchange, e.g. the oxidations of dialkyl sulfides, aralkyl sulfides (but not of the primary sulfoxide products), aldehydes including glyoxylate – most important for control of N distribution in plants as the glyoxylate/glycine couple accomplishs NH_3 transfer there – or of sulfite ion. The small triangular arrows in Fig. 2.14 each denote one electron transfer step, corresponding to passage of 3 e or 2 e or 1 e, respectively. Although the products of these redox transformation include some pretty well-binding, "good" ligands, with nitrite and sulfoxides having rather high $E_L(L)$ values, carboxylates a low one (sulfate does form only weak complexes), binding properties of Mo are changed by this redox process (and possibly a contribution from the N heterocycle, much as seen in Fe porphyrine systems) to an extent that the product is readily cleaved after the reaction. Doing the triangle course around the triple point once means one pathway (closed catalytic loop) through the catalytic cycle. Another effect of macroligand stabilization and dispersion in polymer matrices or additionally at membranes is to stabilize the pentavalent Mo(V) state (which otherwise is unstable up to a tendency towards disproportionation and thus does not turn up in the above Pourbaix diagram); forming a dimer bridged by ligands like in aqueous solution (Kaim and Schwederski 1993; Cotton and Wilkinson 1981; Frausto Da Silva and Williams 2001)

(Gleadow and Woodrow 2000; Gleadow et al. 2003). Both alkaloids and cyanide are probably involved in flux control of certain metal ions inside plants. Due to CN^- metabolism from 2-hydroxinitriles (cyanohydrines) like mandelonitrile along CN^- to SCN^- or to amino acids all of which are ligands but with different $E_L(L)$ values, different binding strengths and selectivities arise. In plants there even is a special pathway of cyanide-insensitive respiration which does not succeed in ATP synthesis but only produces heat so probably has another purpose (Mengel and Zickermann 2007). For young plants, including premature eucalyptus leaves thus toxic to coala "bears", becoming edible for them only later by degradation of CN^-, or root nodules of manioc or "stones" of fruit trees such as apricot or plum CN^- ligands might be a means to locate metals to their sites of uses, with the cyanometallates later releasing essential metals in a controlled manner (cp. Yu and Gu 2009) when cyanide is metabolized. The overall metabolic pathway for metal allocation using cyanide and its precursors may be described like follows (formation and cleavage of α-hydroxinitriles [cyanohydrines] is effected by specialized enzymes in the biota):

$$\text{a) } 6\ \text{R-CH(OH)CN} + M^{n+} \rightarrow [M(CN)_6]^{n-6} + 6\ \text{R-CHO} \qquad [2.26]$$

b) Oxidation of cyanide releasing the metal:

$$[M(CN)_6]^{6-n} + 6\ \text{"S"} + \text{carrier} \rightarrow [M(\text{carrier})] + 6\ SCN^- \text{ (or other products)} \qquad [2.27]$$

Often cyanide is produced by glycine oxidation, allowing to "translate" effects of selective metal sequestration by comparing "mono-" and "bidentate" c and x values for the given metal.

A general matter balance for living beings requires the loss (exit) order of arbitrary essential elements (be them metals or non-metals) to be close to one: over the life-span of an organism, only some small to tiny part of the amount of the element which was ever taken up by this organism will ever persist in "active", vital tissues and retain biochemical function (7:1 or 10:1 ratios for biomass in direct subsequent levels of some trophic chain). Although indispensable for ongoing existence of some species, reproduction also entails loss of catalysts. In addition uptake of essential elements is not fully selective, e.g. Cd may replace Zn, however usually not fulfilling the same functions, and thus there are reasons for damage even though plants possess some protective mechanisms, too, including phytochelatins

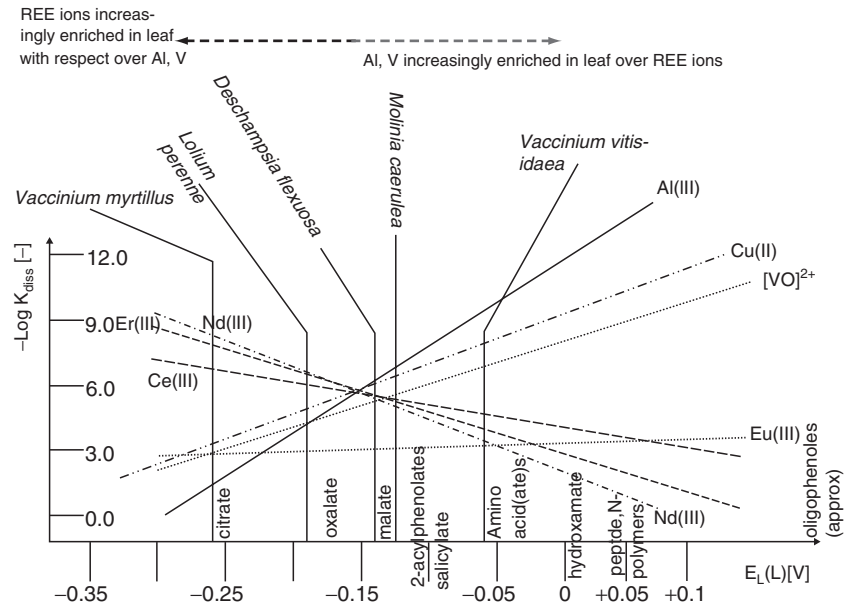


Fig. 2.15 Element fractionations and effective electrochemical ligand parameters for photosynthetic organs of five plant species frequently occurring in forest understorey regions (*from left to right*): *Vaccinium myrtillus* (blueberry), *Lolium perenne* (ryegrass), *Deschampsia flexuosa*, *Molinia caerulea* and *Vaccinium vitis-idaea* (red whortleberry). For other domestic plants, $E_L(L)_{eff}$ values may be higher or lower than the average of the above values, e.g. –0.19 V for each *Lolium perenne* and *Betula alba*. Very low effective electrochemical ligand parameters (oak, dandelion, plants in tropical savanna have even lower ones) cause a relative enrichment of REEs such as Ce, Nd or Er vs Al; Eu behaves differently. Since the lines of complex stabilities of Ce, Nd, Er and Al intersect in the same point [$E_L(L)$ = –0.15 V], abundance correlations of these REEs and Al among various plant species of unlike effective electrochemical ligand parameters are very pronounced ($r \approx +0.9$) while Eu behaves differently, the latter effect probably enhanced by photochemical reduction (see text). The vertical texts correspond to $E_L(L)$ values of ligands which are delivered to soil by plants, fungi or soil bacteria

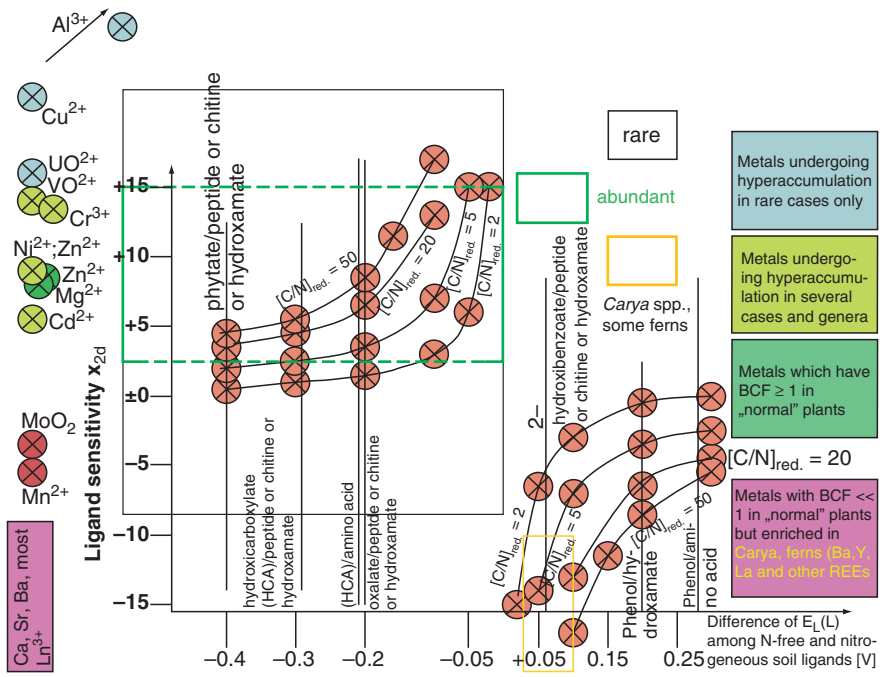


Fig. 2.16 As this nomograph is constructed of the two branches of a hyperbolic function stretching beyond $\Delta E_L(L)_{N;N\text{-free}} = 0$, it can explain non-linearities like maxima in the x_{2d} vs BCF relationship described in Table 2.18. These depend on x_{2d} and soil chemistry, more precisely

Fig. 2.17 Galls growing on an oak leaf. By production of polyphenols the tree tries to cut off the parasite from trace metal supply yet the wasp larva is capable of degrading them, in effect making use of the complexes as a means of metal enrichment. Thus a process used by many kinds of plant (photosynthetic organs and seeds likewise, often based on cyanide/cyanohydrines or on alkaloids) is turned in favor of a parasite (an outcome of co-evolution). Southern Germany, November 2008; photo taken by the author

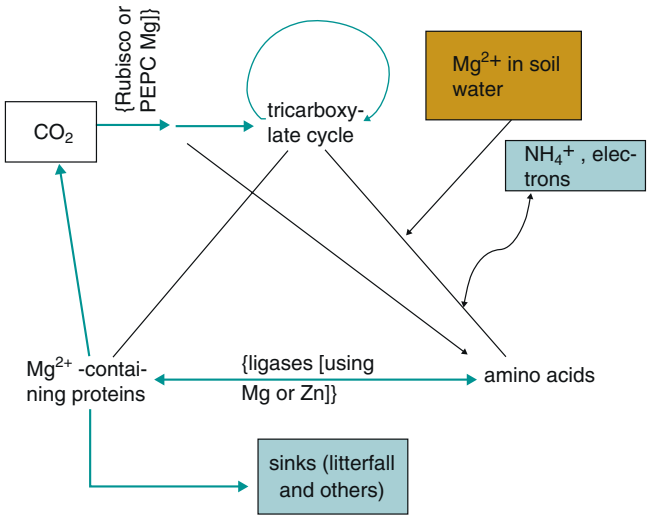


Fig. 2.18 Simplified scheme of the cycle/allocation of magnesium in a green plant. Mg is involved – inter alia – in making peptide bonds, in tricarboxylate cycle, hydrolysis of molecules and binding of CO_2 (ribulose-bisphosphatecarboxylase/oxidase or PEP carboxylase in C_4 plants which also employs Mg or Mn^{2+} in some plants (Kai et al. 2003)). Reaction steps in which Mg takes part as biocatalyst are marked by broken lines/arrows. Citrate and other intermediates of the tricarboxylate cycle, particularly malate, are employed by higher plants for extraction of essential metals, including Mg, Fe and Mn (thus the closed loop) from soil via and by means of the roots. This closed loop depicts a manner of autocatalysis. Amino acids which are required for protein biosynthesis are produced by reductive amination from tricarboxylate cycle intermediates and other 2-oxoacids which likewise eventually originated from Mg-catalyzed (chlorophyll + rubisco or + phosphoenolpyruvate carboxylase) reduction of CO_2, with N reduction starting with NO_3^- as a rule although glycine, like urea, can be taken up directly in some cases even without prior cleavage of the C–N-bond of glycine. While both electrons and NH_3 are required for protein synthesis, they also control and possibly limit growth and reproduction along the "central" (triangular linkage) catalytic loop. Mg-based enzymes or cofactors do not afford electrons directly (chlorophyll transfers energy rather than charge upon excitation), nor are they involved in nitrate reduction (which is instead effected by Mo (first step) and then Fe, Cu). Although Mg-dependent ligases are implicated in biosynthesis also of the proteins needed here, and amino acids can contribute to metal (including Mg) sequestration, this cycle is not plainly autocatalytic

(Strasburger and Sitte 1991) and may cleave or spatially direct heavy metals and other toxic elements (As, Sb) also by means of metallochaperons (Tottey et al. 2005).

For instance, the network may consist of one autocatalytic loop only which is equipped with a number of external "supplies" and "sinks", including or disallowing reversible reactions, or else of some array of coupled, locally linked or nested AC loops. The latter case comes next to the situation in living beings. There is only one way to stabilize autocatalytic phenomena/cycles (including

maintainence of biological key functions such as reproduction), namely, overcompensating these losses by involving the corresponding autocatalytic species (i.e., chemical element) in sufficiently **many** autocatalytic subloops (their number matters, not the relative throughput in terms of either used mass of catalyst or turnover rates). Given there must be some lower limiting number of such functions and there is steady supply from environment or/and "food", this autocatalytic order must at least equal the exit order given by stoichiometries of the various loss processes, better be larger in order to compensate for grassing, natural-cause deaths and mechanical damaging for other reasons. Now we strive to calculate or estimate the sizes of these effects contributing to the exit order. In some higher plant, there will be some first term contributing to exit by steady (lignification, formation of biochemically inactive wood parts, secretion) or periodical (leaf littering) processes which amounts to an exit order of at least 1, reproduction giving rise to one other +1 term. Even if it were neither for predation nor for natural death, the minimum autocatalytic order required the balance the losses thus is ≥2. For an undisturbed autocatalytic system (no exit processes whatsoever), the autocatalytic order O_{ac} is (Clarke 1980; Eiswirth et al. 1991a)

$$R_{cat} = O_{ac} \tag{2.24}$$

with R_{cat} being the number of different (mutually exclusive in terms of different catalyst metastructures) catalytic reactions accomplished by the very element, e.g. Zn in either peptidases, phosphatases or (some) esterases. The sum of exit orders Σ_{exit} is the sum of all the individual exit orders $O_{exit1} \ldots O_{exitj}$; however, strong or critical and thus (in terms of ongoing autocatalysis) sustainable though unstable (for the way of resource exploitation) AC cycles require, regardless of the topology of the chemical network,

$$R_{cat} - \Sigma_{exit} \geq 0 \tag{2.28}$$

with

$R_{cat} - \Sigma_{exit} = 0$ corresponding to a **strong** cycle and

$R_{cat} - \Sigma_{exit} = 0$ to a **critical** one (Clarke 1975, 1980; Eiswirth et al. 1991a,b; Fränzle 2000).

If it were or gets $R_{cat} - \Sigma_{exit} < 0$, the system is bound to collapse in terms of metabolism: it might persist as such without efficient autocatalysis (i.e., attends some "dormant", e.g. anhydrobiotic state) but its matter turnover rapidly approaches zero then. Obviously, this is tantamount to death of an organism or collapse of an entire population on long terms. As shown before, $\Sigma_{exit} \geq 2$ while the number of processes which call for catalysis obviously must be an **integer** one, hence the above condition (2.28)

$$R_{cat} - \Sigma_{exit} \geq 0$$

requires R_{cat} and thus also O_{ac} to be at least 3. Three functions (within one organism of a given species) hence constitute the lower limit for any element to be (remain) essential over extended periods of time. This statement is meant to include (taxonomical, biotic rather than chemical) species which

(a) Undergo thorough changes of their metabolism, environment or nutrition basis during their lifetimes or
(b) Require quite specific compounds (e.g. hormones) to effect such changes, e.g. from tadpoles to frogs with quite some Cu-mediated organoiodine chemistry involved in metamorphosis

This result which was derived here for the first time from SNA determines the chances for any living being (including possible "little green men") to exist on a given array of essential elements. Further on, it is called "**rule of three functions**". These three functions may represent either identical or different biochemical transformations (e.g. hydrolyses accomplished on different kinds of biochemical substrates such as esters, peptides and polyphosphates, mostly executed using Zn^{2+}, or the various molybdenum-based redox processes at aldehydes, sulfite, dialkylsulfides or NO_3^-) on the condition that the individual enzyme must not be able to promote the other transformations, too, e.g. an Mo-based sulfite oxidase should not accomplish oxidations of aldehydes, too (it would not matter if the aldehyde oxidase bears on another metal like Zn or Fe in this case, however), likewise a Zn-dependant phosphatase should not behave as an esterase (again, it would not matter if another, say, Mg- or Fe(II)-based phosphatase (Kaim and Schwederski 1993) would cleave esters, too, as far as the "balance" for Zn is concerned). Even though we restrict the further reasoning to biocatalysis mainly, there are other possible functions, including

- Osmoregulation (provided no substitution is possible (e.g., while potassium is usually considered essential, it can be replaced by rubidium completely in many kinds of organisms, sometimes even reversibly (Scott and DeVoe 1954)))
- Formation of supportive structures in animals and algae (e.g. some foraminifers construct their

exoskeletons from $SrSO_4$ but neither from the Ca nor Ba analogs)
- Chemical signaling (Na and K in nerve cell membranes, Ca^{2+} in cell budding; Fe(II), Cu(I) and other metal ions ligating transmitters such as NO, alkenes in, e.g. regulation of flowering, fruit formation by ethylene (produced by oxidative elimination from 1-aminocyclopropane-1-carboxylate (Habermehl et al. 2003; Mengel and Zickermann 2007)), allyl formate or similar compounds)

The rule of three functions is not fulfilled when substitution is possible without compromising the function (replacement of K by Rb in algae (Scott and DeVoe 1954) and some bacteria (Lwoff and Ionesco 1947), of Zn by Co or Cd in *Thalassiosira* microalgae (Price and Morel 1990)); such systems do not contribute. What about elements which by itself are highly efficient catalysts for sufficiently many biorelevant transformations (e.g. platinum group metals) or are very abundant, with substantial coordinating affinity towards biomass and thus should make their way into biological materials with some potential for executing some tasks, like Al or Ti? Neither of these was so far demonstrated to be essential in any organism. On the other hand, several elements, among them Fe, Zn, Mg and Cu, possess far more than 3 biocatalytic functions in an "average" organism. Except for Cu^{2+} species, complex formation constants do not tend to be very high "inside" proteins ($-\log k_{diss} \approx 4–7$ (Williams and Frausto Da Silva 1996; Vallee and Williams 1968)); thus some share of a metalloprotein should exist as the metal-ion-free apoprotein in the equilibrium state given the low average concentrations of the above metal ions in biomass and fluids except for hyperaccumulators among all plants, fungi and animals. We shall see that, according to SNA arguments, this does not compromise biological function of elements but rather increases dynamic stability of autocatalysis when some, say, 10% of a metalloprotein lack the ion really.

There are other theorems from SNA which bear grave implications for autocatalytic systems, none of which was attributed to such feature of systems stability hitherto although some of the corresponding effects are known for long. Just to give an example, regulation of metalloenzyme activity by dimerization around some semi-open metal center is fairly common, in some cases, particularly with Mo(V), corresponding to the behavior of aquaions or (here) simple complexes in aqueous solution also (Cotton and Wilkinson 1981). Now there is the phenomenon of "short-circuiting" some autocatalytic cycle: as reproduction of one additional autocatalyst entity or substructure from some dimer or oligomer always is less prolific than when starting from a monomer species, any kind of such autocatalyst dimerization brings about a **decrease** of autocatalytic order (Clarke 1980). So, if dimerization is imminent in coordination chemistry as well as bioinorganic chemistry of some element, as with Mo, three functions probably will not do but it takes even more. For example, this more strict criterion will apply to all the metals and non-metals (Si) which readily condense into OH-bridged homo- or heteropoly-acid species, of which at least V and W are essential to many quite different creatures. In addition, these often are accommodated into $Fe_xS_{3;4}$ moieties which then in biochemistry act as all electron sources, redox buffers and "non-innocent" (Kaim et al. 2002) ligands. For Mo this is known to be correct even though the amounts of metal involved tend to be fairly tiny – compared to Zn or Mn or Cu – in animals at least (in plants, they vary over a very wide range). For any macroscopic biological system, but some small part of the essential metals budget within the rhizosphere (for now, further reasoning is limited to terrestrial plants only) is retrieved, let alone put to function, for one part due to unfavorable equilibria in complex formation. Quota of resorption are rarely known either, but one does not require these particular data for modeling (see below) as knowledge of stoichiometric ratios will do (usually M:L = 1).

As pointed about before, the possibility to substitute the central ion of a metalloprotein, say Zn by Co, similarly decreases the AC order of the subcycle under scrutiny (here, that gathering all the transformations promoted by zinc in the given species). In addition, usually the cycles which depend on certain heteroelements (metal ions, Se, B, I) are highly interconnected, with one consuming and using the products of another and vice versa. Perfect, complete coupling of this kind renders all the cycles critical (Clarke 1980) which additionally reduces the chance of effective substitution; accordingly rare are the cases where this really occurs, probably in response to resources becoming scarce (Price and Morel 1990), e.g.

(a) Replacement of Mo by V in nitrogenases of several bacteria (*Azotobacter*; Robson et al. 1986; Rehder

1991) (keep in mind that there is no direct interaction of dinitrogen with either Mo or V – in stark contrast with "model compounds" (polyphosphine/N_2/heteroligand complexes of Mo, W, Re or V exposed to acids) which reduce N_2 to ammonia and/or hydrazine – in these nitrogenases but interaction and reduction occurs in Fe-N_2 moieties. Likewise other substrates reducible by nitrogenase (Eady 2003) such as ethyne, isocyanides, CN^-, N_3^- or cyanamide interact and react via iron).

(b) Zn by Co or Cd in carboanhydrases of some marine algae like Thalassiosira *weissflogii* (Price and Morel 1990).

For zinc the number of functions is in the hundreds, that is, >> 3, even excluding "zinc finger" proteins while the number of functions which are promoted/catalyzed by Mo without a possibility for substitution is low, about five or less while (although more or less obligatory) symbiosis with nodule bacteria such as *Frankia* in *Alnus* (Paul and Clark 1996; Bardgett 2005) or others in leguminoses does not contribute to fulfillment of the three-functions-rule with respect to Mo in the plants: Mo can be replaced (Eady 2003) by V; there are "alternative" nitrogenases without Mo even though these do not occur in root nodules but rather in isolated (self-sustained) organisms like *Azotobacter chroococcum* with a VFe nitrogenase. Both VFe and "Fe-only" nitrogenases are closely related with MoFe nitrogenases but do not arise from the latter by "opportunistic" exchange of metal ions (Eady 2003); the facts that

(a) Mo can be replaced in nitrogenases by other metals, as it is
(b) Not the central ion of them but Fe

means that there is no chance to fulfill the three-functions-rule in, e.g. leguminoses or elder trees if it were not for three functions of Mo independent of N_2 assimilation, e.g. reduction of NO_3^- or oxidation of aldehydes. Conversely, have a look on those elements which apparently are kept from essentiality in autotrophs by consumers, such as Co or V: this implies that photo- or chemoautotrophs must cope without the specific functions of these metal ions. Most noteworthy among the latter are organometal reactions at Co (including reductive alkylations of other metals and of non-metals like As, Sb, Te; catalyzed rearrangements of C backbones) or phenol, aniline halogenations with V, typical products of which accordingly are not accessible to autotrophs and in turn cannot either be exploited by heterotrophs feeding on them. Cobalamins with their fascinating specific chemical abilities must be produced by animals or heterotrophic endosymbiotic gut bacteria rather than plants, V-based peroxidases, haloperoxidases, etc. are available from fungi only. Multifunctional elements like Zn, on the other hand, are not affected by some cases of effective substitution[7].

Although it was that simple to derive the rule of three functions in biochemistry in general, this modification cannot be calculated as straightforwardly because the reduction of autocatalytic order due to oligomerization depends sensitively on (bio-)chemical network topology. In addition to topological aspects of feedback in the network, the exact kind (manner) of dimerization which interconnects identical metal ions needs to be known, likewise the way by which the substrate interacts with this multimetal center (for example, the Mo dimerization effect is also seen with Mo-dependent nitrogenases but N_2 and the other reduction substrates converted by nitrogenase (ethyne, CN^-, nitriles, isocyanides) do not at all interact with Mo atoms but are bound to Fe (which also is the reason why Mo may be replaced by V or Fe)), cp. Clarke (1980). The stoichiometric ratio of metals in some plant or in parts of it is due to multistep and multiple fractionation upon uptake and transport in this very plant, besides the composition of soil, ground or ambient waters; this fractionation involves some couple of rather different ligands, in some cases only delivered if the plant experiences some kind of stress (cp. Fig. 2.9). Then Liebig's minimum principle does hold: that element the administered amount of which is smallest relative to the specific demands of that species will limit functions like growth and reproduction. There is a way in which this also can be interpreted using SNA (see below).

[7] As a rule, there are neither covalent metal–metal bonds nor oganometal species in biology, the only "dimer" kinds directly linked to each other covalently being formed by C–C- or S–S-bonds. Usually, two identical metals are rather bridged by hydroxo-, chloro- or carboxylato ligands (e.g., in haemerythrin), with other μ-ligands like hydride or O_2, N_2 to be considered as intermediates of catalytic cycles or transport tasks. As often metal ions liable to condensation (valve metals, V, Fe) are located close to the "surface" of some protein structure, formation of such dimers will not be precluded by the matrix. Moreover thioferrate clusters also facilitate linking. The thermodynamic and physicochemical (SNA and beyond) implications of this tendency in biochemistry remain to be fully understood.

Obviously, other biochemical effects including reproduction toxicity which likewise depend on metal affinities to some receptor or enzymes can be described in the same manner (Fränzle et al., lecture given in October 2004 at Duzhniki Zdroj, Poland), once again calculating some effective electrochemical ligand parameter, which this time refers to some biological (obstructive) effect rather than mere accumulation. As far as bioaccumulation is concerned, there may be considerable differences even among closely related species of plants like the two *Vaccinium* species *V. myrtillus* (blueberry) and *V.vitis-idaea* (red whortleberry) while there is a typical value, respectively, which holds for many plants which each grow in comparable ((a) Central European, (b) tropic (Ghanaan savanna), (c) temperate-limnetic) climatic/chemical surroundings. The difference between *V. myrtillus* and *V.vitis-idaea* is about 0.2 V (Table 2.7).

Given hapticity (denticity) and electrochemical ligand parameter are identical, a certain ligand – say, the anion of diacetyldioxime – would exert the same discriminating effect on some set of metal ions as the kind of biomass, except for some "amplification" term (see below). This matches the way of metal transport and accumulation in and among different plant organs as described by Clemens et al. (2002), fractionation being due to different values of sensitivity and intrinsic bond stability.

2.2.9.1 The Role of Soil Geobiochemistry and Litter Supply Rates in Effection and Control of Tropical (Amazonian) Metal Cycling: a Perspective from Bioinorganic Chemistry

As was recently done for soil layers below forests in moderate climates (Fränzle et al. 2007), it is feasible to analyze metal cycles in the biota in the thin-layer-humic/high-biomass-per-area regime of Amazonian tropical rainforest also. Generally speaking, the "fates" of chemical elements, including possible maxima in their bioaccumulation by plants or fungi, depend on the kinds or chemical states of ligands delivered by roots or mycelia or soil bacteria to the soil and subsequent chemical and biochemical alterations, which modify, create or remove chemical sites where they bind and retain various metal ions. These all control complexation and in turn retrieval by plants by interactions of metal ions with certain assemblies of N-containing and N-free soil ligands. Eventually "good", "moderate" and "poor" bioavailability properties of metal ions can be assigned to plants growing in given kinds of soil (as defined by pH, redox state, amounts and speciation of non-metals such as P, S, Cl, B), respectively. In the peculiar Amazonian case, an additional criterion to be accounted for is the almost perfect element "recycling" into plants which takes place in this climax biocoenosis and which not even permits substantial amounts of metals to be leached into surface waters even though there is plenty of humic material for complexation and mobilization, conspicuously colouring their waters. Thus Amazonian rainforest, allowing for some additional intake of Mg, Fe by Saharan desert dust admitted by wind across the Atlantic ocean, represents a situation where feedback by leaf litter and decomposition of the latter by fungi mainly keep a set of equilibrium constants for complexes controlling retention as to permit (almost) complete feedback into plants within the stoichiometric ratios required – inter alia – for efficient photosynthesis. This can only be achieved if soil biota – which is confined to a very shallow layer there – controls transformations of nitrogeneous and N-free soil components as to get nearly identical complex formation constants $-\log k_{diss}$ for these different soil ligands. This criterion of extraction selectivity might be met by keeping a difference of electrochemical ligand parameters $E_L(L)$ (Lever 1990) of about –0.07 V, like with malate/amino acid- or 2-α-ketophenolate/hydroxamate or β-diketonate/hydroxamate combinations in soil, root exudates or their metabolites (fungi + humic matter). In addition, the corresponding complex formation constants must be – and indeed are – larger than those with humic (fulvic) acids there. With fungi and soil bacteria providing hydroxamate and peptide ligands copiously, fulvic acids cannot compete for ions. The corresponding equilibrium constants are calculated from the electrochemical ligand parameters according to

$$-\log k_{diss} = x^* E_L(L) + c, \qquad (2.4)$$

with c and x describing properties of a given metal ion, given ligand denticity and metal oxidation state being constant (Fränzle and Markert 2006b). Principally, c and x represent intercept and slope of a regression equation defining element properties.

Keeping this feedback state observed in Amazonia in balance hence requires, besides prolific ligand exudation, an efficient (and rapid) phenol degradation into salicylate-like species (side-chain-oxidation but not ring destruction) on one hand while among N-containing compounds hydroxamates (from fungi, soil bacteria) and peptides should prevail, setting upper limits for hydrolase activities in soil. The other theoretical possibility to account for this perfect recycling would be a difference next to 0 V, permitting indiscriminate feedback of metal ions. Yet, from the set of metals applied for the corresponding enzymatic transformations in bioinorganic chemistry it can be inferred that Fe (key element of oxidizing hydroxylases) and Mg must be readily available and plentiful whilst formation of Zn- and Cu-based (hydrolytic or aromatic-ring-oxidizing) enzymes must be somehow contrived, be it by low amounts or by geobiochemistry, ruling out the second possibility. The model can be tested by analytical data for inorganic and organic components of Amazonian topsoil in both its original and charred (after slash-and-burn agriculture) states. Its additional precondition, besides high and metal-guided fungal and bacterial activities, is that supply of N occurs readily, requiring some abundance of Mo and Co.

In any case, while complex formation constants for root exudates in these tropical environments must be considerably larger than those for soil-humic-matter associates to allow for efficient re-takeup, this must hold for all the essential metals in order to maintain, e.g. Mg/Mn ≈ 5:1 in photosynthetic organs. Therefore, a fairly extreme state of affairs prevails which also has implications for distributions of non-essential elements in Amazonia: which ones might be leached, which (others?) undergo efficient bioconcentration? That is why Y, REEs and Al are recommended as biotracers by this author to follow the corresponding patterns. The corresponding concentrations in soil, plant leaves and outflowing waters give proof of efficient retention towards leaching, suggesting re-takeup by plants and modification by fungi to occur on shorter time scales than washout/leaching were possible during annual flooding episodes. In addition, fungi provide ligands which bind traces of Fe, Cu in a way which renders them hardly accessible to leaching whereas "raw" unmodified leaf litter is more susceptible to leaching of metal ion contents.

2.2.10 *The Topology of Autocatalytic Feedback Patterns in Living Systems*

Autocatalysis is located at the very metabolic "core" of living organisms with their biological necessity (even used to define them as alive, opposing, e.g. crystal growth) to reproduce chemically in an (almost) identical manner. Hence, certain otherwise straightforward catalytical processes might not be applicable to biological systems which reproduce or at least require fulfillment of additional limiting conditions. This statement accordingly is limited to certain, "middle" levels of biological hierarchy as biocoenoses or ecosystems do not reproduce while individual biomolecules (enzymes) or also viruses cannot either without using external assistance. The qualitative differences include

1. Losses of catalyst which limit the catalytic turnover numbers to finite values.
2. An autocatalyst must be obtained from some external reservoir by expenditures of matter (which especially in terrestrial plants may be quite substantial when secreting chelators to the rhizosphere for subsequent retrieval after metal trapping).
3. The very same metal or non-metal must be introduced into different functions: as a result, there are lesser essential elements than would correspond to the number of basic metabolic transformations in the given organism. Therefore, an effective uptake like described in 2) would not suffice but afterwards these elements must be re-distributed and partitioned inside the organism, causing different organs and other autocatalytic subsystems to "compete" for them. Ways of use and amounts of supply must be regulated in a manner as to avoid damage in some of these autocatalytic subsystems, except for part-time inhibition of reproduction.
4. There are additional losses owing to loss of elements from the organism (leaf litter, etc.) or damage by grassing. Even reproduction may contribute to loss when larger substructures are produced like in certain plants or in animals and then separate in reproduction (matter storage organs for embryo development like fruits, oil seeds, root nodules, adventive organs or rather large eggs, placentas).

A general balance for these processes may be constructed when certain contributions are considered

which bring about loss or/and consumption; these were listed just before. As a result of cumulated effects of losses, partial destruction by herbivores and reproduction but a (often very) small part of the matter which once underwent anabolic processes some time during the previous lifetime of the organism still is a part of its living biomass while excretion and CO_2 exhalation in animals, lignification into dead wood and leaf/needle littering in plants remove most of it from active metabolism: for example, until man is grown up at some 20 years and 70 kg of weight, he did consume about 10 t of food (fresh weight, including liquids) which correspond to >1,000 kg of organic C and – depending on the share of meat in the diet – some 50–200 kg of N while he then contains just ≈11 kg of C (organic C, not carbonate in bones and the like) and <1.5 kg of N, about 1% of entire previous intake in either case (after becoming adult, the net gain almost becomes zero, of course). As a result, exit orders are close to one in either case, forcing hypothetical biocatalysts with but one function to vanish rapidly from the population because reproduction renders this a weak autocatalytic cycle, according to the rule of three functions and its formal basis.

Now consider an organism which by some happenstance event of geochemistry finds itself at a site which provides elements which are more efficient in catalyzing one (or two) reactions which are biochemically relevant to this creature, say, an organism which can "run" its hydrogenases (but no other enzymes) with Ru or Pd rather than Fe and/or Ni because it happens to grow next to a PGM *lagerstätte* in contact to topsoil or the rhizosphere. Obviously, H–H bond cleavage and hydrogenations would be much more efficient than with the "original" metals, much as many Zn-dependent enzymes get more active with zinc replaced by cobalt (Vallee and Williams 1968). Primarily one would obtain a more active enzyme, because PGM like Ru are far superior in homogeneous hydrogenation to both Fe and Ni but this advantage could not be maintained over many generations, at least the weak character of such a cycle would bring it to an end much before a specific transport mechanism might be evolved. One could object that PGMs are so rare, $k_i c_i$ being $\rightarrow 0$ for this reason, that essentiality might not be established for this reason and thus in particular PGMs might not replace nickel but there are at least two opposing arguments:

1. Many species, including both higher plants and animals, exist in ecochemical niches like caves or heavily metal-ion enriched springs only which are both much smaller (in some cases, even vertebrate species are confined to some tens of meters) and often very short-lived (black smokers "operate" for but decades) as compared to PGM lagerstätten like the Merenski Reef (South Africa) or Sudbury (Ontario Province, Canada).
2. Cobalt often affords much more active "versions" of metalloprotein after addition to an apoprotein which originally contained zinc (Höhne 1980) while Co abundances in both soil and freshwater (about 1 μmol/L; Markert 1994b) are also sufficient to maintain it.

From this, we will now embark to analyze highly connected multielement systems, that is, take into regard roles of non-metals also and the interactions and dependences among metals.

(How) does a combination of

(a) Tapping some reservoir and autocatalysis (which in plants mainly occurs in leaves and in the rhizosphere) and
(b) The specific autocatalytic functions of different elements

produce a biochemical reaction network of an identifiable topology? Metals accomplish a multitude of catalytic and other biological functions in plants (like in all other organisms) and thus are involved in biochemical (auto-)catalysis, cell budding and reproduction. Figure 2.6 (by Clemens et al. 2002) depicts metabolic pathways used and the speciation and transport forms in both roots and various plant organs. As noted before, SNA singles out feedback loops having autocatalytic features from a larger chemical or biochemical reaction network which prompts us to "translate" the descriptions by Clemens (Fig. 2.6) into an SNA-rooted equivalent which shows reaction cycles and dependencies among the various essential metal ions (Fig. 2.13):

To give an average value, 7.5% of assimilated C (that is, 2.5 times the theoretical value based on citrate) make their way into root exudation (Farrar et al. 2003), with another 6% (rather more in P-limited soils) being transferred to mykorrhiza and the same amount to soil microbes. In the end, 1.5% will reside in the rhizosphere as root leachates while another 1.8% produce

soil microbe biomass and another 4.2% are respirated to reproduce CO_2 which mostly leaves the soil. This balance allows for refining the above estimates:

1. Much less C from assimilation is left behind in the soil for retrieval of metal ions by back-resorption not (yet) accomplished than goes into soil bacteria and mykorrhiza both of which will degrade the complexes and release (or absorb themselves) the metal ions. The total efficiency, allowing for reconsumption of citrate, malate, oxalate, amino acids thus used before in the roots thus is about 20% at best; ≥80% are lost to soil or other local organisms at least in the short time. Yet, there may be almost total (perfect) recycling linked to litter degradation like in Amazonia, allowing for closed metal cycles, too. For avoiding fractionation, the material (both degraded litter and soil(-forming) organisms) must have $E_L(L) \approx -0.15$ V. Accordingly, biomass properties of most kinds of tree leaves or needles in temperature climates (which are almost identical) dictate that ligand donor sites of litter be changed only slightly (if at all) during litter decay but the C backbone be cut down rather when metal recycling be efficient. Which kinds of enzymes – containing which metals? – can effect this?
2. With mykorrhiza absorbing the more plant-assimilated C the less P is present, there is an interesting situation as mobilization of metals (alkaline earths, Zn, Al, Fe(III), Mn(III), REEs) should be the easier as phosphate levels are lower, thus less ligands would be required to retrieve the metals. Mykorrhiza, in turn, consists of heterotrophic organisms (fungi) which absorb a "surplus" of citrato-, etc. metallates, degrading citrate and remobilizing metal ions the more the less phosphate is around. Thus in P-limited conditions substantial M^{n+} becomes available to the plants although by investing twice or more the 3% calculated above.

When analyzing corresponding reaction networks in more detail, as will be done for Mg afterwards (Section 2.2.12), the electrochemical ligand parameter and its changes must be considered once again to understand transformations. For now, this is not yet feasible for fluxes of a multitude of essential metals just because informations on the involved chelators are missing; this precludes the kind of analysis done for Mg before, notwithstanding issues like redox speciation. As, moreover there is neither an idea concerning chelator denticities (maximal or actual) or binding sites (thus $E_L(L)$), it is thus outright impossible to estimate or predict relative stabilities of complexes involved in transport or biocatalysis and thus the share of metals being kept from an active state.

Already SNA representations of rather simple (one or two kinds of autocatalyst only in the entire reaction network; Eiswirth et al. 1991b) AC feedback systems show that it makes a decisive difference whether there is a sink for autocatalyst effectively cleaving parts of it beyond limited diffusion losses or not. For example, with the famous bromate/CH-acid based chemical oscillators of the Belousov-Zhabotinsky kind, onset of oscillations can be caused or terminated by sweeping Br_2/bromine oxides by gas (nitrogen, argon) bubbling or increasing their amounts by photoredox processes. Thus (at least sometimes essential) elements like Mn, Ba and often also Fe, being precipitated along with birnessite/manganite, apatite must be distinguished in biochemistry strictly from others where such sinks (insoluble [aqu-]oxide-, phosphate or carbonate phases) do not usually occur under environmental conditions.

There may be other elements where essentiality goes along with a potential for substantial losses from biomethylation, especially Se and As, causing another sink: via exhalation of polymethyls or methylelement hydrides (Thayer 1995) or $E(CH_3)_n^+$ (n = 3 for Se and n = 4 for As) excretion autocatalysis will be controlled. Possibly, biomethylation is so efficient in reducing conditions that many of these elements were going to be vented before getting a chance for biological function (the other elements subject to biomethylation are highly toxic (e.g. Sb, Tl, Hg, Te) or become so [Sn, Bi] upon biomethylation rather than being essential in methylated or some other speciation forms). There is no established role for any of these organoelement compounds in a biochemical C-transfer cycle (possibly catalytic/enzymatic oriented to product biosynthesis) except for some Co (cobalamine), Ni and As species besides the familiar sulfonium salt S-adenosyl methionine. Then, however, both cobalamine and S-adenosylmethionine must be very ancient species (simpler Co complexes act much like cobalamine also (cobaloximes, Schrauzer 1976) and conceivably would have been precursors to the biosynthetically most demanding cobalamin B_{12}).

2.2.11 SNA and Metal Transport in Terrestrial Plants

We are concerned with terrestrial plants (and their consumers) mainly throughout this work although certain aquatic plants demand some consideration for their sometimes "exotic" element essentialities (Ba in desmides, Cd in alga *Thalassiosira weissflogii*), but there is another reason for closer scrutiny: because the "roots" of aquatic plants are for spatial fixation in sediment only and do not take up substantial amounts of trace metals, it is far more straightforward to link concentrations inside the plant and in the water immediately surrounding it than for soil in terrestrial plants. When land plants reach ages of up to several millennia, there are also sinks *within* the organism, while establishment of (dynamic) equilibria with their environment (here: soil) takes much longer than in aquatic plants. As noted before,

- Sinks are fundamental features in autocatalytic systems according to SNA.
- Essential elements may even become depleted upward a trophic chain, eventually limiting its length, moreover.
- The behavior of some reproducing system will change in the very moment when a former resource turns into a sink for the same – essential or non-essential – element, e.g. by biogeochemical changes in the area. In either case further existence or at least the ability of the species/population to keep on reproducing are jeopardized. Effects in population structure can be anticipated even if all the individual organisms survive at first.
- Diffusion barriers can massively influence, initiate or abandon non-linear chemical processes.

Yet the description given so far is a "semistatical" one insofar as it is concerned with complex formation constants and with the distribution of elements among different organs (it is interesting to note that, as a rule-of-thumb, the number of metal ions per cell and biological/biocatalytic function is rather constant in humans: about 5×10^5 atoms (somewhat less than **1 attomol/cell*"function"**, except for vanadium). However, organisms are open systems and metabolism usually entails a permanent flow of matter in- and outward of the cell or entire organism. In addition, reproduction implies an increase of biomass with some constant (except for temporary changes before or in metamorphosis) chemical composition, thus the trace elements must be taken up from some external reservoir steadily while the entire process is autocatalytic. To achieve this, reservoirs must be "tapped" by secernation of some chemicals (ligands delivered to soil by all bacteria, fungi and plant roots, digestion) and the elements thus taken up be converted into some functional speciation form(s) owing to conservation of matter. During this, losses are unavoidable and the biosynthesis of the corresponding sequestration agents of course requires additional expenditures of matter (e.g. C, N fixed before in photosynthesis and nitrate reduction) and of metabolic energy. Although Fe(III) or Cu(II) form extremely stable complexes with biosequestration ligands, the maximum uptake efficiencies are some 70–80% owing to the fact that there are ligands and other binding partners (humic acids, other polyphenols, oxide, sulfide, silicates including clay minerals) in the soil also which likewise react efficiently with the above ions, bringing about considerable **retention**. Though the components used to take up and transform all the essential element (-speciation forms) are thus parts of some highly complicated autocatalytic system, thus require binding regularly, there are also loss pathways directly linked to metabolism:

In plants and animals, these include excretion with but partial retention of essential elements, leaf or needle littering, binding of trace elements to dying parts of wood (sometimes to considerable concentrations) and of course consumption by eaters. Obviously, structures like single kinds of molecules (metalloproteins) or entire organs can only be maintained if regains of essential elements from the environment (in animals and other heterotrophs: + food) will at least compensate for the losses. If there is cell budding or reproduction, the demands and loss orders will even be higher. This translates into formal, calculable criteria, to be derived from Stoichiometric Network Analysis (see below). This fact poses limiting conditions similar to those imposed by Gibbs's phase rule and will be discussed now.

There are various concentration and other chemical gradients inside a plant, and there is stimulation of gradient formation and some uneven distribution (see Sterner and Elser 2002 for the various parts of an apple tree or the autoradiographs on certain elements in different plant species (Figs. 2.7 and 2.8)) will cause trapping of some elements somewhere in the plant, e.g. transport may be stopped at the root/xylem (shoot)

interface region. There is another important feature concerning the relationship between autocatalysis (which implies to have some outer (re-)source for the autocatalyst or its precursor(s), thus the system must be open with respect to matter exchange) and biology: if autocatalysis is combined with an open structure, and there is some kind of storage, the system is capable of **undergoing Darwinian evolution** (Schuster 1984).

Generally speaking, a substantial part of the metals taken up via the roots by some tree or scrub is bound to end up in either litter or in the wood fraction of trees which, although it does still support the photosynthetic organs in the merely mechanical sense of the word "support", will no more participate in metabolism. Therefore but a small part of metals remain in a state accessible or active in biocatalysis over extended periods of time after being taken up. The exit order which results from this deposition into wood and litter is close to 1 at least but will be much larger in polyfunctional metals like Fe, Mg, Cu or Zn. Moreover, the average lifetime of an enzyme molecule – let alone the residence time of its central metal ion if there is/are one(s) – will be much shorter than the lifespan of any multicellular organism regardless of ligand exchange rates at the central ion itself, hence repair mechanisms by e.g. chaperons are most important to limit losses. To a first approximation, the residence time $\tau_{resid(M)}$ of this central ion in either metalloenzymes, in molecules like chlorophyll, amavadine or the siderophores which are not catalysts in physiological conditions (unlike amavadine) will be the timescale of water self-exchange $\tau_{H2O/H2O}$ at the metal ion (Frausto daSilva and Williams 2001; Jordan 1994) times the complex formation constant, that is,

$$\tau_{resid(M)} \approx \tau_{H_2O/H_2O} \times 10^{[x*EL(L)+c]} \qquad (2.28)$$

or

$$\log \tau_{resid(M)} \approx \log \tau_{H_2O/H_2O} + [x \times E_L(L)] + c \qquad (2.29)$$

Typically, $\tau_{H2O/H2O}$ ranges from 10^{-8}–0.1 s, with $-\log k_{diss}$ somewhere between 5 and 7, rarely 10–12 (Williams and Frausto Da Silva 1996). If, then $\tau_{resid(M)}$ in a metalloprotein or cofactor (e.g. molybdopterin) is smaller than the lifetime of an organism or its individual cells (which is of order 10^3 – several times 10^8 s), mobilization and defunctionalization of metals otherwise involved in biocatalysis take place if $-\log k_{diss} < 11$ and corresponding substrate and protein/cofactor side chain $E_L(L)$ values (even if the modes of reaction of the biogenic complex differ from that of the simple aquaion). Accordingly, metal-ligand bonds are fairly labile rather than extremely stable in both transport systems and functional proteins (Vallee and Williams 1968; Höhne 1980; Williams and Frausto Da Silva 1996). Losses thus happen on a steady basis, and equilibration also occurs in cases of homoeostasis (possibly except for Cu and Zn). Essentiality must thus be "organized" with larger amounts and flow rates of the elements as if the metalloproteins, etc. were really at least kinetically stable towards hydrolysis, attack by non-substrates or protein degradation. This conclusion is backed by the difference between structures and complex formation constants of cytosol proteins in leaves or needles where the metals end up for the time being vs those values which hold for the fairly simple organic ligands in root secretes. It is established for long that distributions of certain metal ions already in subterraneous plant parts **cannot** simply be attributed to effects of single simple ligands such as citrate or malate (Farago 1986).

The ratios among C, N and P in an organism are the "classical" parameters of ecological stoichiometry (Sterner and Elser 2002). These three non-metals are all involved in binding metal ions to biomass:

- **N** forms quite a number of ligand kinds (amino acids, carboxamides [Asn, Gln, urea, peptides, hydantoins]), including carbamidines (Arg), imidazoles (His), purine- and pyrimidine bases all of which directly bind to metal ions.
- **C**, on the contrary, binds metal ions usually only by/after forming oxoanions (carboxylates), except for cyanoligands (cobalamine, hydrogenases), CO, alkenes (binding to Cu(I) in plants), isocyanides (marine sponges) and some ligand species which are almost restricted to anaerobic organisms (alkyl groups at cobalamine Co, Ni-F430 porphyrine and CO/acetate synthetase).
- Similarly, **P** will bind to metals by oxoanion species (phosphate esters, nucleic acids); there are no M-P bonds like built those up by phosphines, and, beyond classical ecological stoichiometry.
- **S** can be more abundant than even N, e.g. in iron-oxidizing bacteria (Fränzle and Noack 2008), with cystein and methionine residues more capable of binding metals than sulfate ions.

Now we are to determine which of these four non-metals prevails in metal biocomplexation, taking into account the

(a) Relative concentrations of the non-metals, given by C/N, C/S and N/P quotients, and
(b) Relative complex formation constants

C/N in higher plants is 7–30 for the entire plant but 20–50 for photosynthetic organs, N/P uses to be 50–80 and C/S about 500 (stoichiometric ratios each). Both S and Se are partly bound into ligands (cysteine, methionine, selenocysteine, dimethyl selenide and many plant components such as those of *Allium* species including garlic), but likewise partly in weakly complexing anions like SO_4^{2-} or SeO_3^{2-}, respectively (total S in leaves or needles is about 3 mg/g DM or 90 mmol/kg; C/S ≈ 450 and N/S ≤ 20).

Because complex formation constants depend on $E_L(L)$ and c, x by Eq. 2.4, one must reconsider the list of "bioligands" and their denticities in Table 2.2 plus the above concentration ratios, corrected for the fact that, while a majority of N-, P- or S-sites behaves as a ligand, the C backbones of proteins or carbohydrates will not. An effective C/N ratio thus is smaller than the value reported above, and so is C/S while N/P (and N/S) can be used "as is". Now there are typical ranges of $E_L(L)$ associated with these elements, rather narrow for P and C, somewhat larger with N and very variable – from thiolates to sulfoxides – for S. Assuming N/P = 50 and N/S = 20 in higher plants (rather than [N/S] < 1 in *Leptothrix ochracea*), P (-oxo) or S binding will only prevail over complexation of metals at N sites if $10^{-\Delta\log k_{diss}} > 50$ (P/N) or >20 (S/N), that is, $-\Delta\log k_{diss}$ must be more than 1.70 or 1.30 (larger) with respect to competing N-donor sites. The formal treatment once again is obtained by rearranging Eq. 2.4 appropriately, like follows

$$-\log k_{diss} = x\, E_L(L) + c, \tag{2.4}$$

accordingly

$$\Delta \text{ Log } k_{diss} = x \times E_L(L) \tag{2.30}$$

or

$$\Delta \text{ Log } k_{diss}/x = x \times E_L(L) \tag{2.31}$$

or

$$x_{nd} = (\text{or} \leq)\ \Delta \log k_{diss}/\ \Delta E_L(L) \tag{2.32}$$

Table 2.13 contains the pertinent values for all the biologically (and in quantitative terms) relevant P-oxo-, C-oxo-, N- and S-ligands including nucleic acids, from which the following differences can be obtained.

Both complex stability constants and structural investigations such as X-ray crystallography prove generally bidentate coordination of phosphate esters or pyrimidine bases (due to the linear structure and donor site arrangement of the latter, no higher complexation to a single metal ion is possible, much like with gallic acid, caffeic acid and similar polyphenol compounds) while for NAs an average $E_L(L)$ of −0.30 V was calculated by this author from complexation equilibria (data in Izatt et al. 1971; Massoud and Sigel 1988). If phosphate(-ester) ligands can dominate over N-donor sites with their $E_L(L)$ values between −0.05 V (amino acids) and +0.12 V (imidazol) for the more abundant chelators, its $-\log k_{diss}$ must be more than 1.7 higher ($10^{1.70} = 50$) (the abundance of both S and P in biological materials is sufficiently large as to allow for establishment of such equilibria in higher plants; C/N about 30 and N/P = 50, that is, C/P about 1,500 (and C/S ≈ 450). Although both values are smaller than $C/\Sigma_{metals} \approx 200$ but, leaving aside Ca, both P and S are about as abundant as the heavy metals which meet the conditions to be calculated). Obviously such selectivity depends on the ligand sensitivity x – more exactly x_{2d} – of the metal ion as shown in Eq. 2.26. Equation 2.26 and the above $E_L(L)$ differences then yield a "necessary" $x_{2d} \leq -4$ to have P(oxo)ligands prevail over N and of −4 to −12.5 for C-oxospecies.

Table 2.13 Estimates of element fractionation due to $E_L(L)$ ranges associated with different ligand donor- or donor support (backbone) atoms. For C, an average value of −0.21 V is assumed

Element	Range of $E_L(L)$, kind of ligands	$\Delta E_L(L)$; C/element [V]	$\Delta E_L(L)$; N/element [V]	$\Delta E_L(L)$; P/element [V]
Carbon	−0.30 (carboxylate) to 0.40 (CNR)	0	About −0.20	About −0.10
Nitrogen	−0.05 (amino acids); +0.03 (carboxamides including peptides) to +0.36 (RCN)	+0.16; +0.24 (peptides)	0	About −0.30
Phosphorus	Around −0.30	+0.09	About +0.30	0
Sulfur	−0.54 (thiolate) to +0.47 (sulfoxide-S)	−0.33 (cys residue), up to +0.68	−0.53 (thiolate) to +0.48	−0.24 up to +0.77

Accordingly, among the essential elements which are rather abundant in biomass this does hold only for Ca and Mn while all others will be located at N- or S-binding sites. Other elements which should prefer P(-oxo) sites and thus interact with the "P switch" for proteins, include heavy alkaline earths Sr and Ba, REEs except for Sm, Tb and Eu (both oxidation states in the latter case) and tetravalent Ti, Zr and Hf (Table 2.3).

There is a role for the most abundant among these elements, Ca ($x_{2d} = -10.98$) in plants also which directs refers to P(oxo) site interactions: by addition to certain parts of nucleic acids, Ca^{2+} causes uncoiling of DNA and thereafter translation and expression of certain parts of the genome. REEs are known to stimulate cell budding and growth of plants, which is exploited in agriculture in China and Canada, while Ti(IV) (budotitan and similar cytostatic agents (Fränzle and Markert 2003 and literature quoted there)) acts by modifying the P switch activation or desactivation of enzymes. Thus these ions with x_{2d} between −8 (Ba) and some −60 (Ti(IV), extrapolated from data for Ti(III); omitting hafnium which chemically is known to behave most similar to its lighter homologue Zr, but considerably differing with respect to x_{2d} (Table 2.3), have very limited biological roles except for Ca and Sr, causing us to omit the 4th group transition metals from Fig. 3.1, rather magnifying the principal part around the "window of essentiality" (Ti, Zr, … are way below the depicted parts of this diagram). As c and x depict subtle differences among closely related elements (REEs in general, Y/Ho, Zr/Hf, Nb/Ta) the biochemical relevance of this kind of parameter readily explains why, e.g. rats do readily locate and thus separate Zr and Hf into different organs and tissues (Dulka and Risby 1976)) all conform to the expectations from the above reasoning on partition to various sites: chemically similar elements may be separated while there is very strong correlation among others (for example, V and Al).

Of course, stoichiometry of metals with respect to N and P is also involved and influences the effect. While N/Ca ranges from 5 to 17, P/Ca ≈ 1, which implies that changes of "free" Ca concentrations can indeed control biochemical activities of phosphorylated (or to-be-phosphorylated) biocompounds in an efficient manner (Sr and Mn might do so also in special conditions, the others are too rare as a rule). For both limnetic algae and freshwater higher plants (Lemnaceae) $E_L(L)$ values are higher than with temperate-climate terrestrial plants (or at least for photosynthetic organs of the latter). Figure 2.15 illustrates the relationship between differing biochemical properties – which strongly depend on taxonomic features in plants – and ecophysiology of some typical representatives of understorey plants (grasses, scrubs) in Central European forests. As noted before, $E_L(L)_{eff}$ is not just an outgrowth of the kind(s) of ligands delivered by the roots but these values are thereafter modified by metabolic processes and membrane diffusion (once again including coordinative interaction) (cp. subsequent part 2.2.11).

Even when there are rather few different phases/organs coexisting, it is feasible to "distribute" biochemical functions among different parts inside one given organism (the multitude of such biochemical functions related to one single biocatalyst – e.g. Mo – is an outgrowth of the rule of three functions directly derived from SNA (see Section 2.2.7 above). Chemical difficulties related to the rule of three functions are relieved in such metazoans also as different organs or tissues can also differ with respect to their inner chemical conditions, which allows for promoting unlike chemical transformation in the same organisms by one given element: either

1. Redox differences among the organs permit to organize metabolic redox catalysis close to different triple points of a complicated Pourbaix diagram (say, with V, Mn or Mo as biocatalysts), with three different redox states "rotating" around this triple point while promoting external redox reactions quite efficiently because the three states coexist in that triple point (say, with nitrate reductase or different S-center oxidases containing Mo) (Fig. 2.14) or
2. Different binding environments (ligands, protein matrices, vacuole liquids, exo- and endoenzymes in soil and biomass) can alter the position of such triple points sufficiently when salt concentrations or pH values differ also

that various, unlike reactions can be catalyzed, fulfilling the rule of three functions with respect to the corresponding redox-active bioelement (Mn, Fe, Mo, Cu, etc.): metalloproteins or peptides tend to bind small molecules like chloride, citrate, simple amino acids, etc. to the central ion and Ca^{2+}, REE ions and others to outer parts of the protein molecules. Complexation in the first-mentioned way changes the redox potential, shifting the position of triple-points and the general shape of Pourbaix diagrams, often even changing their topology if the common three elements M, H and O

Fig. 2.19 (**a**)–(**c**) A serpentinite soil site in France (Massif Central) and the site-typical vegetation: ferns (2.19b), including extremely metallotolerant species. Ferns, specialized flowers and some scrubs (2.19c) take over while woodified plants are absent. September 2006, all photographs by the author

are supplemented by N, Cl, P and S (Figs. 2.14, 2.19 and 2.20). Unless complexation directly brings about disproportionation, the triple point within some Pourbaix diagram will move rather than vanish (Fig. 2.14), e.g. for Mo or Fe (Fig. 2.19), but as redox catalysis is most efficient next to some triple point, other substrates are turned over at substantial rates than before. In biochemistry, this effect is amply used for both molecular recognition (e.g. with redox-active signalling ligands such as NO) and selective activation; consider, e.g. the dependence of both sensitive substrates and redox potentials [Cu(I/II)] in the various Cu-containing redox enzymes also mentioned in Table 1.1. With molybdenum, another most important central metal of redox proteins, *pterin* is a cofactor which both keeps Mo dissolved in aqueous medium and modifies (increases) the redox potential Mo(VI/V) or Mo(VI/IV) owing to the significantly positive $E_L(L)$ value typical for N-aromatic heterocycles (here, +0.25 V). Tungstoenzymes (Johnson et al. 1996), however, are not simply varieties of molybdoenzymes doing the same jobs in highly acidic and/or very hot environments their bearers (typically, Archaea or Clostridia) live in, but promote quite different reactions at far lower redox potentials, like oxidation of formate or aldehydes or activations – including hydrogenations – of CO, ethyne and similar substrate. There also is no W-dependant reduction of dinitrogen although there are fairly many W(0)complexes which accomplish this reaction owing to the fact that biological N_2 reduction in nitrogenase is unrelated to Mo but takes place at a Fe site, with Mo replaceable by either V or Fe itself. In other systems than nitrogenase, however, the Pourbaix diagram shows how and why Mo enables reductions of nitrate or sulfoxides.

The latter reactions can be catalyzed efficiently (fast kinetics owing to substantial reduction of the activation barrier) if the coupled redox system,

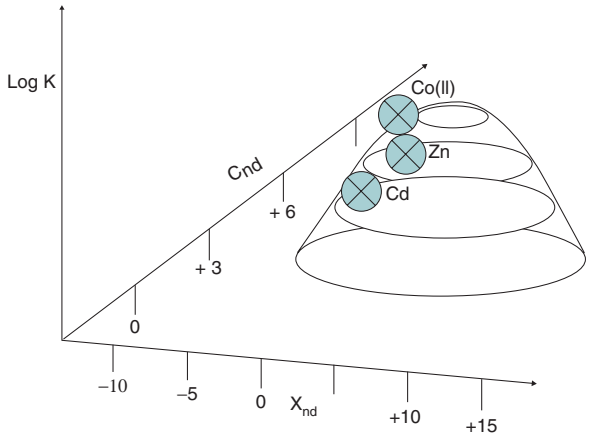


Fig. 2.20 Formal fitness landscape of various biocatalytic and inactive (i.e., the latter being located outside the "window of essentiality") metal ions for the carboxypeptidase A (Vallee and Williams 1968). The enzyme reconstituted by cobalt (treatment with EDTA, then addition of Co^{2+}) is considerably more active than the native Zn "version", while Cd (and other ions) afford poorly to non-active metalloproteins *in this case*. (Relative) catalytic turnover rates are from Vallee and Williams 1968, x and c values: this work and previous publications by this author. Here, sufficient abundance of the corresponding ions is taken for granted owing to in-vitro addition to apoprotein, so $c_x k_x$ is not limited by c_x but merely represents the catalytic features pertinent to k_x, obtained from either biochemistry (several metal ions being present, e.g. in phosphatases) or experiments with reconstituted apoproteins

e.g. oxidations of aldehydes, of sulfite or As(III) or reduction of nitrate, is close to such a triple point, in this case to one in the modified Pourbaix diagram of molybdenum (Mo-based enzymes generally contain two Mo ions (Kaim and Schwederski 1993)). Two functions, however, would just provide a theoretical minimum for a system reproducing by cell budding or other (derived) kinds of asexual reproduction since such an organism, depending on just critical cycles for autocatalysis, would be sensitive towards arbitrarily small perturbations. There are many perturbations of that kind, including

- Loss of some catalyst directly via the surface of the biochemically active parts of the organism (e.g. sweat, formation of and element deposition in some kind of bark, reaction of metal ions with humic acids and other DOM compounds causing "leaching" from the organism, especially in aquatic animals
- Partial inactivation of an enzyme by destruction of protein, loss of the activating metal ion (if there is some) or substitution of the latter by toxic "alien" components, and finally, which is less obvious
- Dimerization of metal-containing enzymes or formation of multienzyme complexes

By association of additional ions to otherwise very similar metalloproteins thus the same biochemical redox system (same metal in a protein center) can promote transformations of different substrates, sometimes augmented by chemical gradients or other differences among the various "phases" or organs, thereby fulfilling the rule of three functions.

Theorems from SNA, based primarily on topologies of reaction networks, state that any kind of chemical interconnection between two or more autocatalytic core species brings about some kind of "shortcut" in the reaction network and separation of subloops of lower autocatalytic order which entails a "weakening" of the AC character of the system, generally speaking, a reduction of AC order (and often also increased losses (exit orders), cp. CB and CS cycles derived from the former (Eiswirth et al. 1991a,b)) (Clarke 1980). Among the so-called valve metals most of which tend to form both homo- and heteropolyacids, up to an extent that few monomeric Mo(V)-species can be maintained after reduction of Mo(VI) or oxidation of Mo(III; IV) species (Cotton and Wilkinson 1981; Riedel et al. 2004), V, Mo and sometimes W are involved in biocatalysis. Mo enzymes indeed behave like this in redox cycles, furthermore the protein matrices tend to coagulate ("coacervates"; Oparin 1947) as these macromolecules bear surface charges and H bridging groups on the surface (amino acid side chains of serine, asparagine, arginine, lysine, etc., sugars in glycoproteins). Unless locked up in a rigid membrane or other kind of matrix as single, isolated molecules, proteins will interact and partly coagulate, which sometimes is a requisite for their functioning also, e.g. with many Mo oxidoreductases (Kaim and Schwederski 1993).

Though the rule of three functions might be fulfilled by different kinds of enzymes bearing the same metal ion and operating on the various diastereomers of some polychiral substrate, e.g. of threonine or leucine, more frequently one single element in an enzyme will "distribute" the required three functions over two or three different basic reactions/transformations, e.g. some among the about 30 listed in Table 1.1. Due to this, elements which might promote lesser of the above different catalytic transformations in biomass because of their "exceptionally" high or low x values, either cannot bind quite different substrates or get rid of the correspondingly different products. Thus their

chances to activate different reactions are much smaller and they can hardly fulfill the rule of three functions. Using c and x parameters in Table 2.3 which describe the above relationship including Sabatier's principle for optimum catalysis depending on $E_L(L)_{substrate;product}$ and the list in Table 1.1 (the column dealing with "optimum" catalyst ions), one will see immediately that the corresponding number is very small for both Al and Ti, in fact zero for Ti ($x_{2d} << -50$ for the tetravalent state), that is, Ti, as abundant as it be on Earth, is not an efficient biocatalyst (Table 2.14).

Even the ease of retrieval or possible photocatalytic uses or such in alkene synthesis from the carbonyl compounds principal in biochemistry (McMurry reaction) apparently cannot compensate for this as Ti is unable to bind the primary substrate. For Al, Zr or Ti abundance cannot replace catalytic versatility with respect to *various* functions, that is

$$\Sigma k_i c_i \ (n_i = 3 \text{ or larger}) \rightarrow 0 \qquad (2.33)$$

It does not matter that essentiality of some other elements (Na, K, Ca, Sr, Si) – if it actually refers to a certain element which cannot be replaced – will be unrelated to biocatalysis: e.g., K can be fully replaced by Rb in many organisms (Scott and DeVoe 1954; Lwoff and Ionesco 1947), Ca roles, e.g. in control of cell budding can likewise be fulfilled by Sr and by various REE ions as a rule, while there are specific functions (unknown up to now) for Sr in corals and certain algae and for Ba in desmides, biocatalytic or not. Thus, neither K nor Ca are fundamental as single elements in such organisms. Examples for such different reasons of essentiality include electrolyte balances, changes of membrane permeability or protein shapes (Ca (and pathological: Ba) action on tertiary structure of actin and myosin fibers in muscles, to be relieved by Mg^{2+} as a "better" complex center) or their contributions to supporting structures such as algal skeletons ($SrSO_4$ in some dinoflagellates). Here, catalytic flexibility is low or non-existent, testified by extreme values of both x and c.

How will the situation change if water gets scarce around some metal center, e.g. in lipid-dominated environments (e.g. membranes, micelles, fatty organs) or deep inside protein molecules or multienzyme complexes? The relative stabilities of metal complexes, e.g. the Irving–Williams series, do hold for many – even rather non-polar – non-aqueous organic (Golub and Köhler 1979) and inorganic (Waddington 1972) solvents also; hence, although the absolute values of c and x for a given metal ion will change, corresponding to another zero-point given by solvolytic properties (e.g. with strong donor solvents like DMSO, CH_3CN, amines, formamides or NOCl) or/and stronger π-binding behavior than water (nitriles once again). Moreover, Brønsted base activity (removing protons from an acid to convert it into some ligand) is usually much less pronounced than that of water, mostly combined with lesser ionizing capabilities. The latter ionizing capability is almost non-existent in ester solvents, representing lipids. Thus an extrapolation on media in which lipids prevail, e.g. to membrane-bound proteins, is feasible, stating that, although the c and x values will be changed the basic criteria of a catalytic cycle still hold, including the condition that the product be more weakly bound than the educt. In ester solvents hydride transfer mediated by metals like Al or Ti (or by boron) could take place without hydrolytic problems, or some

Table 2.14 Number of efficient catalysts for principal biochemical transformations sensu Table 1.1 vs c and x. The display given here is not identical to Fig. 3.1 as it is focused on specific "optimally-catalyzed" interactions/catalyst ions rather than unspecified – and sometimes far-from chemical optimum – biochemical essenti ality. Nevertheless, a similar inner range of versatile catalysts in biochemistry can be discerned

x_{2d} (to the right); c_{2d} (downward in this column)	<–40	–40 up to –25	–25 up to –10	–10 up to 0	0…10	10…20	20…35	>35
0–2								
2–4				5 (Mo) + 2 (Mn) = 7	5 (Co)			
4–6					8 (Fe)[a] + 6 (Zn) = 14			
6–8					3 (Ni)	3 (V)		
8–10							9 (Cu)	
10–12								

[a]Two out these eight are iron-thioclusters like in ferredoxines

organometal reactions could be exploited by biology which as a rule are avoided. Hence, although such "manipulations" are conceivable, the very high or low x_{2d} values and respective ligand (substrate) selectivity in metals like Al, Ti or Zr obviously preclude sufficient catalytic versatility. This does not change even though the absolute values of c and x will differ with respect to those in water (cp. Table 1.1 which includes data from organic solvents concerning "optimum" catalysis which yet does not relieve the obstacles to the above metals), and lesser solvation will bring about some catalytic "deshielding" ("phase transfer catalysis" (PTC), "nude ion effect"; Makosza 2000). Hence in spite of this well-known phenomenon the various k_x remain too small although the tissue concentration c_x will be really large for both Al and Ti, somewhat less so for Zr.

2.2.12 Stoichiometry of Terrestrial Plants and Its Implications According to SNA

As shown before, SNA provides a blueprint for a coherent description of biochemical use and transport of elements on various hierarchical levels up to entire ecosystems (Fränzle 2000); given both the structural complexity (with lots of unknown or unidentified actors at microbial levels at least in any such (eco-) system which probably yet constitute a substantial part of both biomass and turnover contributions) and the difficulties to define outer boundaries of such a ecosystem, it is probably wise not to attempt a "complete" description including biochemical reaction kinetics (like that done by Schilling and Palsson 1998 for *Escherichia coli*) and population dynamics but rather restrain to qualitative yet theorem-backed arguments from SNA. The latter qualitative arguments nevertheless give rise to certain quantifying statements, the most important among them being the rule of three functions. Considering that

1. Autocatalysis is a necessary but in various ways chemically precarious precondition for life which additionally poses some critical constraints of matter use in life and reproduction, and
2. There is a direct relationship between the stability of typical (containing the ligand sites also involved in biomass-metal ion interactions) metal complexes which extends to both the dynamics/intermediates of some (bio-)catalytic cycle and the variety/width of metal ions in proteins

many chemically plausible agents of catalysis cannot be applied in biocatalysis exactly because reproduction brings about additional constraints to keep such agents involved in biology over many generations ending up in evolution. Presumably, this is the reason – or at least one principal reason – why there are so many differences between the "optimum" catalysts as judged from the experience of the technical chemist (optimal ones even when working in physiological or similar conditions) and those actually employed in biocatalysts (enzymes) even after almost four billion years of biological evolution (Table 1.1). Making some bilance of trace metals in biology hence must include the autocatalytic features of biochemistry, represented by reproduction, and the above limiting processes: catalysts (enzymes) are both synthesized and destroyed on a regular, steady basis. Besides outright destruction of some (metallo-)protein it may also be inactivated by the "phosphate switch" or by loss, removal or exchange of some central metal ion.

Now turn to a complete plant as it chemically interacts with the surrounding ecosystem (generally speaking, an ecosystem is a man-made concoction for purposes of separating some regionally and chemically constrained biocoenosis for reasons of simplifying closer analysis; yet, as it is both meaningful and well-introduced to speak of pond ecosystems, deciduous forest ecosystems and so on, and some chemical exchange features can be used to distinguish these ecosystems from those supported by other biocoenoses, we happily follow these terms, prompting us to speak of the above plant/ecosystem chemical exchange modes): in roots metal ions, anions and water are abstracted from soil solution and several mineral phases (Figs. 2.6 and 2.13); the ligands from the tricarboxylate cycle are both small and not too selective. This partial system is autocatalytic with respect to Mg and Fe since enzymes containing these metals are in turn involved in Krebs cycle and thus afford the species then tapping external Mg and Fe reservoirs. The further way was already discussed; suffice it to say that water transport driven by evaporation in the canopy brings about concentration, the more so as the volume (living biomass) of canopy photosynthetic organs is considerably smaller than the complete volume

(and water mass) of the rhizosphere. Hence, as the equilibrium is shifted towards metal complexes upward in the plant, increase of metal ion concentrations becomes a spontaneous chemical process if $-\log k_{diss} > -1$, and this even holds for metals like Ba. With cytosol metalloproteins being more stable than citrato- or malatocomplexes, transport is enhanced additionally. As $E_L(L)_{malate}$ is (calculated to be from complex stabilities) −0.13 V, less so for citrate, and all the biorelevant metal ions except of Mn(II), Ca and Sr have positive x_{2d} values, it is straightforward to assume that $E_L(L)$ for the carrier proteins be larger than this value, implying a role for ligand sites other than carboxylate (aspartate, glutamate residues). However, bear in mind the changes are accomplished by just removing the primary (sequestration) ligand in the manner of oxidative exchange. Hence, although x_{2d} is positive for most of the metals, $E_L(L)$ of secondary carriers (notwithstanding equilibria between bi- and polydentate behavior towards some of the metal ions which might occur in a (carrier) protein, often around −0.05 V (for Be, Mg, Al, Fe(III), etc., cp. Tables 2.5 and 2.6)) need not be larger than −0.13 V. The two diagrams 2.16 (above) and 2.24 show the consequences of littering. It is interesting that almost all hyperaccumulators most of which enrich Ni, contain very high levels of histidine in xylem liquids (Farago 1986). Free histidine bears an imidazol moiety ($E_L(L) = +0.12$ V) but, like other functionalized amino acids, its complex formation constants with various metal ions are very similar to that of simple glycine or alanine, suggesting an identical way of binding (Kiss et al. 1991). Possibly in hyperaccumulators there are peptides, which like albumin (two imidazols, two carboxamide functions at the same one terminus) contain much histidine in a position exposed to metals.

Whereas we here deal with biocatalytic processes within a single organism mainly, this line of reasoning can be extended to collectives of organisms and matter flows therein (Fränzle 2000 in Breckling and Müller (ed.)). The formal link through trophic chains or networks is represented by the concept of "consumer-driven nutrient feedback" (Sterner and Elser 2002) borrowed from ecological stoichiometry, here to be extended from C, N and P to a multitude of metals and additional non-metals like S, B or Si. Of course, when comparing the autocatalyst-(re-)producing processes to their antagonists or reasons of losses, the overall balance may be "positive", zero or "negative", with the latter case being a blueprint for disaster.

A "negative" saldo, which means losses by degradation by death, apoptosis or consumption prevail, causes decrease of biomass, in extreme cases down to extinction of the population or entire species, while sufficient autocatalysis/reproduction at corresponding levels of trace element supplies means an increase of biomass (positive saldo). Neither pathway is sustainable in the long term, the latter over-exploiting the resources (we saw before the actual limits set to Earth's biota in incorporating the essential elements from those layers of Earth which are accessible, with only few ones incorporated to >1% after four billion years of evolution) while the former means extinction, probably without evolutionary successors. In between those two extremes there can be another way of element accumulation and use behavior, at least over much of the life-span of some organism, which means about equilibrium between uptake, autocatalytic uses and loss pathways can be established: net zero. Obviously, this state is less unstable than the other two ones. In a vital organism, there may or may not be ongoing production of living biomass over the life-span: reptiles tend to grow throughout their livespans, fishes may also do so but most members of social (both territorial and swarm-forming) species are kept from growing on by social status/rank while many fungi steadily increase extent and biomass of their mycelia. The other case is with insects, amphibians, birds and mammals which have well-defined maximum sizes which they attend long before death; often they will not reproduce much before actually being grown up. In wood plants, living biomass may be far smaller than dead wood which fulfils mechanical (supporting) functions only anymore and moreover is almost devoid of other essential elements than C, H, O, Na and Ca, for example, C/N ranges from 200–600 (rather than 6–30 otherwise) there (Sterner and Elser 2002), except for some trees enriching oxophilic metals in wood (Al; Y and light REEs in *Carya* trees (Fränzle 2008)) which yet are not biocatalytically essential. Thus in the latter kinds of organisms uptake of biocatalytic elements is limited to steady-state almost as a precondition for "diverting" some of it to for reproduction: they must "switch" from internal growth/bioaccumulation and synthesis of biocatalysts to producing offspring rather than accomplishing both at a given time. Concerning metabolic energy throughput and the expenditures required to maintain it, it is noteworthy that this effect is not limited to homoiothermic animals but extends to amphibians and higher plants

(with insects, it may be related to the manner by which metamorphosis takes place). Zn, Fe and Cu are key-catalysts there, but by now it is unknown whether levels of these elements are different in the "steady-growers" or how they are allocated there as, for example, all fish ("steady-growers") and amphibian (size-limited) eggs might take up essential metals from ambient water continuously, with their egg shells being most suited for it while those of birds obviously cannot, and metal ion permeabilities of reptile or monotremata eggs deposited in or on soil are unknown though conceivable.

Non-wood plants will sometimes grow extremely large at certain sites of high humidity and plentiful trace element availability from volcano soils (Ruwenzori Mountains, Azores Islands), suggesting an influence of trace metals which are more abundant there on gene expression and thus growth regulation.

Hence a quantitative comparison of processes which form (from uptake of components over gene expression to "assembly" of the corresponding enzyme(-system)s) and consume/divert/destroy biocatalysts can be taken to characterize the state or "situation" of some biological system using SNA. If autocatalysis (AC formation) is dominant, the corresponding catalytic cycle is called "**strong**", if the autocatalyst is consumed by so many or efficient ways that it is bound to vanish in the long term, this is referred to as a **weak** cycle (obvious enough though it might persist outside the realms of autocatalysis) and in the above "pseudo-equilibrium" state there are **critical** cycles (Clarke 1975).

"Equilibrium" here means that the net production of new autocatalyst is just balanced by the sums of loss orders of loss processes; this also occurs if two AC cycles are fully coupled with one using the products of the other as autocatalyst and vice versa ("hypercycle"). All three kinds of autocatalytic cycles may be constructed in very different ways since both production and cleavage of autocatalysts can occur in various manners. With weak cycles, the end of autocatalysis is imminent, but strong cycles are likewise unstable because their matter demands is high and bound to increase steadily. Yet, critical cycles may also display non-linear behavior, e.g. explosive growth, chemical (or population) oscillations or bistability if they are perturbed by additional factors (Eiswirth et al. 1991a). Therefore SNA arguments give proof that ecosystems or biocoenoses are but (at best) metastable, can never be inherently stable, regardless of the ways in which essential elements are used, whether there is net inflow or loss from this system or not, etc. because it is impossible to construct some metasystem (i.e., the biocoenosis) from reproducing structures (living beings) only when implying linear behavior (steady-state condition) or else the involvement of weak cycles: any way to try to fix "steady-state" behavior in a biocoenosis thus will bring about both changes of the material basis of autocatalysis and destruction of certain agents, reduction of biodiversity and eventually evolution counteracting what was tried to establish: this is exactly what was observed in *Biosphere II* (Arizona). Accordingly, the state which was dubbed "labile equilibrium" before just is less unstable in terms of kinetics of change; there is nothing like some stable or indifferent equilibrium. The same happens when coupling different biochemical cycles inside a single cell (Clarke 1980); when different critical cycles are made to co-exist in biochemistry they will "slowly explode" (Clarke 1980) or/and are bound to evade each other spatially: this brings about budding of cells. But what about those elements like C, N, S and metals Zn, Mg, Fe, Cu which have literally hundreds of biochemical functions in any one species and hence should give rise to strong cycles? Though chemical oscillations are known to occur in vitro in multienzyme systems, e.g. in glycolysis, they are rare in "actual" biology: we observe critical cycles to prevail in biology/biochemistry, with the loss processes precisely compensating (another way to grant for critical behavior is to produce strong cross-wise coupling among the cycles). It should be pointed out that SNA theorems provide general rules for dynamic consequences of allocation and use of matter in autocatalytic systems which do not depend on any chemical details; rather it is feasible to construct, e.g. oscillators by selecting appropriate autocatalytic reactions for integration into a given network which otherwise would just display a clock reaction or even "boring" linear transformations. How, then, essential elements are "selected" (retained in biological evolution after these were once endowed with some biocatalytic function, e.g. by exchange) and what might limit their number, leaving no function in any known organisms to highly abundant elements like Al, Ti even though there might be bioaccumulation? Theorems from SNA can thus moreover show how (and why) some early "choice" of biocatalyst may become "frozen" even beyond thorough geochemical changes.

Obviously, any loss/export of essential elements is going to hinder autocatalytic processes, while some part of the components taken from soil by roots are just

used to synthesize compounds needed to open this very resource (balancing data in Farrar et al. 2003; Bardgett 2005) which is a sideway of autocatalysis; for example, citrate ion serves to mobilize Fe, Mg, ... in and extract them from soil as citratocomplexes while these very metals (and Mn) are involved as components of metalloproteins in the tricarboxylate cycle which affords (inter alia) citrate ions. Thus the contribution of some chemical element to some autocatalytic cycle cannot become or be made arbitrarily small if it is to persist in the autocatalytic network. As will be shown, "arbitrarily small" means not necessarily a certain amount of material but a certain number of functions (>1, anyway) which must be exerted, a number of which can and in turn will be counted.

This kind of autocatalysis creates a two-way relation: ligands are used to obtain and transport certain metal ions (in fact, most of them), and these very metal ions are required to produce these ligands. Side-reactions such as formation of "parasitic" complexes or salt phases (which plants or fungi can no longer resorb), or irreversible "escape" of organics into soil and its heterotrophic organisms will shift the M/C ratios and yields. Obviously, one can only balance these processes as long as some range of concentrations and concentration ratios is maintained; this ratio is accessible to calculation or at least estimate from some combination of ecological stoichiometry and quantitative complex chemistry. As an example, let us consider the ratio between Mg and some principal ligand component (like C), that is, that ratio $Mg:C_{citrate}$. The same reasoning holds for coupled biochemical processes at least if they dominate the biochemical features of the kind of organisms:

- In green plants, photosynthesis couples water oxidation (accomplished by Mn) to the use of the electrons thus released for CO_2 reduction after binding to phosphorylated sugars (ribulose-1,5-bisphosphate, or phosphoenolpyruvate which is not exactly a sugar but readily forms oxaloacetate upon Mn(II)-catalyzed reaction with HCO_3^-); for all steps of the latter transformation chain (phosphorylation of sugars by kinases, CO_2 transfer to the product ribulose-1,5-bisphosphate by *rubisco* and eventual reduction of C_4 intermediates) Mg is required. Hence, to get and maintain a balanced electron flow without loss to hydrogen release (like often occurs in photosynthetic cyanobacteria) or oxidation of biomass or external organics rather than water by PS II it is mandatory to keep some constant Mn:Mg ratio. This latter Mn/Mg ratio should be observable in photosynthetic organs of different plants (fig. 2.26), leading us back to the Biological System of Elements.
- In animals, metabolic energy is stored by phosphorylation (done with Mg in kinases) after oxidation of nutrients, effected by enzymes using Zn or Mo or Cu (oxidoreductases).

While in theory, given we (would) know the turnover rates and metal contents of the corresponding enzymes, the M/M′-ratios required to balance the above reactions in either animals or plants can (could) be calculated, we are better off by now just considering that the organisms which can be analyzed now (as they exist plentifully) apparently keep these and other metabolic balances which depend upon metal ratios – otherwise they would not be here. If, e.g. Mg:Mn (Mg being a highly abundant soil cation, and Mn the second-most abundant heavy metal after Fe) in soil matches the ratio required for efficient photosynthesis, there are still two conditions at least one of which must be fulfilled:

1. Either, in reducing soils with mobile Mn, the uptake efficiencies (BCF values) must be identical, corresponding to a certain $E_L(L)_{eff}$ of about −0.07 V (*V.vitis-idaea*, various limnetic plants) or
2. In oxidizing soils, Mn is transported in the Mn(III) state which latter is capable of attacking soil-borne organic polymers including wood and lignite

Concerning, e.g. a tree or some other terrestrial plant which obtains its metals from soil mainly, when the ratio in local soil is given, BCF values must be balanced also in order to get efficient photosynthesis – or the excess metal must be removed or stored in some way. In oxic ocean water Mg:Mn exceeds the "proper" value by many orders of magnitude (Mg^{2+}: 53 mmol/L; Mn (total) some 0.4 nmol/L (Nozaki 1997), that is Mg:Mn ratio $>10^8$ rather than about 5). SNA arguments concerning maintenance of some stable flow state and thus coupled supply of two parts of collective autocatalyst can be linked to relative complex stabilities.

Yet we are left with some problem: as a rule, kinetics of the individual enzyme reactions are not known; moreover, for enzymes of fossil organisms they cannot be known at all unless the sequences are highly conserved. So we must apply some non-numerical representation of SNA to account for the differences in patterns of essential metals observed among different larger groups of contemporary organisms.

In metazoans, autocatalysis with all its structural and dynamic features (Pota and Stedman 1994) additionally provides a basis for **signal transmission**: during cell-budding, concentration waves of Ca^{2+} ions propagate over cell membranes, acting as a means of both information and control, and they are released from storage proteins. Reproduction, as considered from the point of view of periodical autocatalytic use of certain kinds of matter and the limitations due to it, is a really critical process which excludes elements with just single, isolated functions in biocatalysis to become or remain essential, however efficient they may be for this isolated "purpose".

The smaller number of "phases" in plants and proterozoans corresponds with the lower number of essential chemical entities ($n < 20$) to yield a similar number of degrees of freedom. Nevertheless, this restricted set of different tissues can give rise to rather different internal chemical conditions, partly due to photochemical charge separation in leaves or needles. During photosynthesis, both strongly oxidizing (O_2) and reducing compounds (sugars, hydrogenated quinons) are formed. The presence/production of magnetite nanoparticles, formed from Fe(III) in all leaves, needles and moss leaves (Fränzle et al. 2009), gives proof for considerably reducing conditions in leaves even though dioxygen is produced there.

Since metal distributions in plants may be quite different and sometimes even inhomogeneous within photosynthetic organs, and there is continuous fractionation during upward transport in a terrestrial plant, an analysis of effective electrochemical ligand parameters and their ecochemical implications is required. Thus climatic effects or uncommon soil chemistries or pedochemical impact of succession which also alter soil chemistry and redox conditions will cause parts of a plant, e.g. the photosynthetic organs (this work and Fränzle 2007; Fränzle et al. 2008) or microroots (data for beech roots: Tyler 2004b), to respond, either within range of adaptation or by being replaced with other species having different (intermetal, C/S-, C/N-) ecological stochiometries and $E_L(L)_{eff}$ values: in B. pendula at least, there is no influence of being grown on peat, "common" soils or sewage sludge whatsoever concerning $E_L(L)_{eff}$ values.

The following analysis of different parts of pine trees (Table 2.15) from "an old alluvial sandy soil in

Table 2.15 Concentrations and BCF values of various parts of Scots pine (*Pinus sylvestris*) trees

Metal[a]	1-year-old needles[b]	Older needles	Branches	Knots	Bark	Wood	1-mm ø roots	5-mm ø roots
Al	400 (142)	200	400	120	230	7	1,430	82
	0.040	0.020	0.040	0.012	0.023	7×10^{-4}	0.143	0.008
Co	0.9 (0.124)	0.8	0.6	0.2	0.4	0.1	0.1	0.7
	0.45	0.4	0.3	0.1	0.2	0.05	0.05	0.35
Cr	4.8 (1.72)	4.0	1.6	0.8	1.0	0.3	0.9	0.6
	0.074	0.061	0.025	0.012	0.015	0.005	0.014	0.009
Cu	4.2 (4.28)	2.5	3.0	1.2	2.0	0.6	3.5	1.2
	0.38	0.23	0.27	0.11	0.18	0.055	0.32	0.11
Fe	150 (118)	370	650	78	100	5	7,171	46
	0.0075	0.019	0.033	0.004	0.005	2.5×10^{-4}	0.36	0.0023
Mn	430 (208)	740	430	185	123	61	134	50
	2.0	3.4	2.0	0.85	0.57	0.28	0.62	0.23
Ni	6.0 (1.4)	2.1	1.1	0.3	0.4	0.3	1.1	0.4
	0.55	0.19	0.10	0.27	0.36	0.27	0.10	0.36
Ti	15	30	25	6	15	1	46	6
	0.015	0.03	0.025	0.006	0.015	0.001	0.05	0.006
V	0.6 (0.65)	1.2	1.8	0.8	2.8	0.2	0.6	0.5
	0.007	0.013	0.02	0.009	0.031	0.002	0.007	0.006
Groups of metals with equal BCFs	Fe, V and Co, Cu (Ni)	Al, Fe and Cu, Ni	Cr, Ti, V	Al, Cr and Co, Cu	Co, Cu and Cr, Ti	Mn, Ni and Co, Cu	Cu, Fe and Co, Ti	Ti, V (Al) and Al, Cr
$E_L(L)_{eff}$	a. –0.02	a. –0.22	–0.03	a. –0.11	a. –0.20	a. –0.26	a. –0.31	a. –0.02
	b. –0.20	b. –0.18		b. –0.20	b. –0.05 (–0.03)	b. –0.11	b. 0 (–0.01)	b. –0.11

[a]Assuming the following metal concentration levels in soil: Al 10,000 µg/g, Co 2.0, Cr 65 (mainly $FeCr_2O_4$), Cu 11 (average for podsols and sandy soil[s] in [former] USSR), Mn 217 (like with Cu), Fe 20,000, Ni 11 (like with Ni), Ti 1,000, V about 90

[b]For comparison: values by Markert (1996), obtained in Lower Saxony, given in round brackets

Ukraina" (Kabata-Pendias and Pendias 1984) gives a first idea as to how much $E_L(L)_{eff}$ might vary with different parts of a (coniferous) tree (*Pinus sylvestris*).

The differences between one-year-old and older needles in *Pinus sylvestris* and other coniferous trees were noted elsewhere (Fränzle et al. 2008); the considerable difference between very small and thicker roots obviously has a role in metal transport. There are two groups of $E_L(L)$ values, one near 0 V for branches, larger roots and young needles and another, considerably more negative for the other parts of the plant. Apparently fractionation among the former organs is avoided, while bypassing some internal sink (wood) in transport: that $E_L(L)_{eff}$ is less negative in bark than in wood but similar to the values for needles means there is no general fractionation between wood (from where metals can be remobilized hardly) and photosynthetic organs while pine bark has a higher affinity for positive-x_{2d}-metal ions than pine wood, thus including most essential elements, keeping them in metabolically active (and conducting) regions of the stem. Total removal of bark will spell death for a tree while wood probably would form just a sink (and does so with accumulation of negative x_{2d}-elements in certain other trees at least). So there is a chemical gradient between bark and wood which effects some fractionation against such elements which are not required up in the needles, the only problem being associated with Mn ($x_{2d} = -5.32$ in divalent state) which is required in photosynthesis and amply present especially in older needles. Here oxidation to Mn(III) is known to facilitate transport in plants and animals alike so it probably would not be mislocated to wood which shows Mn contents as low as those in larger roots but less than half the value in bark and about 10–15% of those in needles.

Cobalt and titanium are assumed to be present as Co(II) and Ti(III), respectively; estimate for Ti(IV): $c_{2d} \approx +6$; $x_{2d} \approx -80$. It is obvious even at first glance that metal fractionation differs in the different parts of Scots pine, including metabolically (almost) inactive materials (bark, wood) which is likely to reflect complexation differences among various kinds of lignin and other supporting materials. Note the extreme difference in Fe levels between small and larger roots and the fact that Co levels are smaller in small roots, contrary to all the other metals.

For higher trophic levels and phenomena other than metal fractionation, analysis of entire organisms was done on certain aquatic animals (Fränzle and Markert 2002b, 2006a; see Table 2.16 below). While biomagnifications of heavy metals including REEs has no perceptible effect in undoped waters here, toxicological and reproduction-toxicity data are available for a variety of metal ions including REEs in the latter case (Weltje 2003). Reproduction toxicity should be related to gonad cells, inhibition of swimming to muscles and/or nerves whereas bioaccumulation refers to an entire animal, usually. Accordingly the different biochemical properties of the organs or differences between certain organs and the average gives rise to three different values when consistently using c and x data for bidentate binding.

While all three aspects of biological metal ion-organism interaction can be tackled by Eq. 2.4, for both light and heavy metal ions, the $E_L(L)_{eff}$ values thus obtained differ by a considerable 0.30 V between biomagnification and reproduction toxicity. Although toxicological uses of the "heavy-metal" concept date back to 1904, it is hard to find any term from quantum chemistry which links a density value of $\rho \geq 6$ (or 5) g $\times$ cm^{-3} for the elemental metal to chemical details of metal **ion**-protein interaction which are pertinent to toxicology (Fränzle and Fränzle 2002). Hence this familiar term is of no use for understanding links or differences among different features of metal ion-biomass interaction; neither are light metals (Be, Mg, Ca, Sr,

Table 2.16 Effective electrochemical ligand parameters, kinds of metal ions on which the calculation is based (there are no omissions for reasons of "mismatch") and biochemical effects in *Daphnia magna* (Fränzle and Markert 2006a). For a denticity of 2, consistent values were obtained in all cases. The rightmost column is for illustration only and not meant to suggest that these ligands are directly involved in the above (column 1) effects of metal administration. The values were derived from regression analysis of toxicological activities or BCF values (Weltje 2003) after rearrangement of Eq. 2.1 for the electrochemical ligand parameter

Kind of biological/ biochemical activity	Denticity [–]	Effective electrochemical ligand parameter [V]	Metal ions used for regression	Biorelevant ligand types (examples)
Bioconcentration	2	–0.25	Various (alkaline earths, 3d-, Ln^{3+}-ions)	Hydroxicarboxylates, phosphorylated sugars (e.g. phytate)
Death (inhibition of swimming activity)	2	–0.08	Various	Amino acids, oxaloacetate, etc.
Blocking reproduction	2	+0.05	REE ions (Ln^{3+})	Peptides, other carboxamides

Ba, Al, [Ga], Sc, Y, Eu, Ti, [V]) generally distinguished by "extreme" c or x values nor can pronounced toxicities of heavy metals like Cd be attributed in this way except for "abnormally" high c values. The generally positive sign of the latter value (c_{2d}, except for Eu^{2+}) renders a link between reproduction toxicity and metal ion addition to nucleic acids unlikely.

The grossly different values for the two closely related *Vaccinium* species which usually do not occur next to each other hence are probably results of different metal acquisition strategies rather than due to the competitive exclusion principle: both plant species differ in spatial distribution rather than in take-up of resources (for example, both are rich in manganese while the differences can be seen in pairs of other metals).

Of course, analytical data concerning the composition of plants (Garten 1976; Markert 1996; Kabata-Pendias and Pendias 1984, etc.) or some parts (organs) thereof translate into stoichiometric ratios among the elements. Using quantitative data on contributions of and from photosynthetic C-fixation, which must be "invested" for retrieval of essential metal ions from soil via roots, an estimate of the extent of organic ligand production and transport coupled to transport of either essential and/or abundant (e.g. Al^{3+}) becomes feasible. A matter flow balance like that used in SNA modeling depends on such an estimate. For the M-sequestering and –transporting ligands the following values of C/M hold:

(a) In citrate- or malatometallates: 6 (citrate) or 4
(b) If associated to phytochelatin (n = 8), glycine terminus; 1–3 metal ions (Dorčak and Kręçel 2003: C/M between 22–66)
(c) In metalloproteins (e.g. such in leaf cytosol): C/M about 500

When citrate ligands already transporting metal ions are reloaded to the tricarboxylate cycle, the metals will be relocated to other coordination environments in very much the manner of **oxidative ligand exchange** which will be outlined somewhat for its importance in introducing less strongly bound ligands in biochemical systems also: classically, oxidative ligand exchange (OLE) is replacement of one ligand which is easily oxidizable (or sensitive towards photooxidation; Fränzle 1992, 1996) by another which usually is just produced by this redox electron transfer or obtained from the surroundings. In the former case, there is a bound or oxidized form of the new ligand presented to the precursor complex, like in reactions such as

$$[Fe(CN_6)]^{3-} + 3Br_2 \rightarrow [FeBr_4]^{-} + 2\,Br^{-} + 3\,(CN)_2 \quad \text{(with iron remaining in Fe (III) oxidation state)} \qquad (2.34)$$

or

$$[OsCl_3(CO)(ox)]^{2-} + Cl_2 \rightarrow [OsCl_5(CO)]^{2-} + 2\,CO_2 \qquad (2.35)$$

where the (otherwise far more) strongly coordinating ligand cyanide can be replaced by bromide because cyanide is converted into the weaker cyanogene ligand which in turn cannot compete with either anion Br^-, or CN^- for Fe(III). Here, the product of the redox reaction is accepted by the metal ion in its entirety while in other cases, e.g. during comproportionations of a ligand in its colloquial negative oxidation states and some non-metal halide, only parts will be transferred, e.g.

$$[M_{soft}ClI_5]^{2-} + IF_5 \rightarrow [MClF_5]^{2-} + 3\,I_2$$
(+ fluorochloroiodometallate intermediates;
Preetz & Thilmann 1993 for M = Re) (2.36)

Thus, making use of (the rather mild oxidant) iodine pentafluoride, iodide can be replaced by fluoride even though soft class B metals strongly prefer iodo to fluoro ligands, hence there would be no reaction of the above iodo(-chloro)metallate with either HF or alkali fluorides.

In either case, the free energy released in the redox process more than compensates for the loss of binding strength by ligand exchange, that is:

$$17.0\,\Delta\varepsilon \times k_e > \text{xnd} \times \Delta\,E_L(L) \qquad (2.37)$$

with 17.0 being the relation of logarithmic equilibrium constants from free energy changes in redox chemistry (0.059164^{-1}) at RT, $\Delta\varepsilon$ referring to the difference of redox potentials and k_e being the number of transferred electrons.

Here (in or directly above plant roots), citrate is removed by oxidation (tricarboxylate metabolism) and weakened in its donor abilities; thus xylem peptide or protein carriers can take over. This is because (a) citrate is no longer present after its metabolic degradation and (b) the later ligands in the citrate cycle are poorer (since bidentate rather than tridentate) metal (ion) acceptors than citrate. Thus citrate gets transferred to these (alas, yet unspecified) other ligands (Figs. 2.6 and 2.13). As this process is effected by citrate oxidation at least in the forthcoming steps it is fully analogous to the above instances of oxidative cyano or iodo ligand exchange by bromine, chlorine or IF_5 reagents, with NAD^+ or – eventually – O_2 acting as electron

sinks without simply being converted into the new ligands. In biology, another very straightforward case of oxidative ligand exchange giving rise to metal translocation inside a plant case is **oxidative desamination** of amino acids (often of glycine, proline, or glutamic acid) which likewise take part in metal sequestration and thus are converted into poorer ligands, with N used elsewhere. Glyoxylate is so weak a ligand that even glycinatometallates with M having "extreme" x values will undergo this transformation readily.

After that the water flow transports the complexed metal ions upward in the shoots, eventually into photosynthetic organs where parts of them will be converted in metalloproteins like rubisco or PS II Mn_4Ca system. If ligands become involved which form complexes which may be absorbed by roots but thereafter are not subject to biological ligand oxidation for reasons of their structures precluding it (e.g. EDTA which cannot undergo oxidative transamination as it is a tertiary amino acid (Fränzle et al. 2005)), the modes and ranges of biological metal transfer may change: e.g., in natural conditions Cd^{2+} uses to remain mostly in the roots (Fig. 2.7), not penetrating into the xylem and beyond in larger amounts (for example, average Cd levels in leaves of plants investigated by Markert (1996) were close to 0.1 μg/g, with Zn/Cd ≈ 800 (by mass) rather than 64 in soil (Kabata-Pendias and Pendias 1984; Emsley 2001)). If, however, the soil is exposed to EDTA or its salts, Cd readily makes its way into shoot and even leaves (Sapundjieva et al. 2003; Schwitzguebel and Porta 2003), paving the way for (thus assisted) Cd phytoremediation by plants like corn (*Zea mays*). The reason for this is that, owing to lack of in-root ligand biooxidation, the rather stable (Mizerski 1997) Cd-EDTA species remain intact all the way up the shoot and water flow (Noertemann 1999). Metal transfer by the above oxidation of primary (sequestering) ligands thus can create a bottleneck to further transport, especially in cases where there is a distinctive preference for ligands such as citrate, e.g. with REE ions[9]. As a result, the ratio citrate/metalloprotein must be much smaller than [C/M] itself, as can be seen in Table 2.17 for photosynthetic organs.

Table 2.17 Concentrations of some essential metals + V, Co (not required by plants in general), Al and Pb and their stoichiometric ratios towards C for quantifying treatment of the ligand expenditure required for metal sequestering (or effecting uptake of unwanted elements such as Pb; data recalculated from Markert (1996) and Emsley (2001)). Nitrogen in higher plants has but a minor rule as an element providing M-trapping donor functions, which is quite different in grasses and even more so in fungi (carboxamides, hydroxamates; Kaim and Schwederski 1993). Likewise, nitrogen donors are imminent during further metal transport in higher (vascular) plants, prompting also to consider the N content. Here, the Mg/Mn ratio of about 5 in terrestrial plants appears again, referring to coupling between both branches of photosynthetic redox equivalent transfers. Consider the rather constant Mn/Mg ratio implied by linking both "branches" of photosynthesis (PS I and II)

Element	Amount (mmol/kg DM)	[C: element] [–]
C	40,000	1
N	800–2,000	20–50
Mg	30–50	800–1,300
Ca	120–150	ca. 300
V	0.01	4×10^6
Mn	5–13	3,000–8,000
Fe	2	20,000
Mo (nitrate reductase and others)	0.001–0.007	6×10^6–4×10^7
Co (essential only for few plants, often linked to symbiosis)	0.002	2×10^7
Ni	0.02	2×10^6
Cu	0.05–0.08	5×10^5–8×10^5
Zn	0.3–3	1.3×10^4–1.3×10^5
Al (not essential)	5	8,000
Pb (does block porphyrine biosynthesis)	0.01–0.07	6×10^5–4×10^6

[9] As this "problem", which e.g. precludes transfer of Sm^{3+} and several other REEs into wheat grains almost completely (that is, down to detection limits, Emsley 2001), occurs likewise with all the REEs and other "citratophilic" metals (Al, Ga) in a given plant species, non-fractionation (identical BCF, here thus small, usually BCF < 0.02 for these metals in terrestrial plants) does imply that effects occurring elsewhere during respeciation cancel each other or do not differ either. Thus the determination of an effective electrochemical ligand parameter based on identical BCF among these and other elements **remains valid**; in addition, multi-element approaches provide rather identical $E_L(L)_{eff}$ values for the same plant analyzing REE and transition metal BCF data (cp. Table 2.19).

The ratio between C and the metals essentially is that among C (in ligands like citrate) and the sum of the three most abundant (albeit weakly, yet complex-forming) metals Ca, Mg and Mn, making $C/\Sigma_{metals} \approx 200$ in higher plants; as might be anticipated, marine algae growing in some 0.05 M Mg^{2+} medium contain more of it, decreasing C/Mg to about 100. Alkali metals except of Li are reluctant to form complexes and thus can (must!) be neglected here. The ratios among amounts and thermodynamic stabilities of metal ions in several parts of some plant which are connected by transport processes and biochemistry, determine both concentrations in certain parts of this plant and – according to SNA – stabilities of all the autocatalytic part- or subsystems.

All the systems are coupled by chemical reactions, there is abstraction of matter in every case or subloop due to this coupling, and accordingly the holo- or metasystem which invariably depends on matter uptake from the environment or foodstuffs and likewise cannot avoid losing some matter. That (coupling of inheritance and autocatalysis by **chemical** processes) exactly is what distinguishes living beings from growing crystals (which likewise absorb matter from the environment to produce predetermined structures), "plain" autocatalysts (which lack structural information to be reproduced) or from robots which can assembly their very likes (Von-Neumann-devices; though there formally is reproduction, the coupling between assembly (a mechanical process presumably accomplished by electrical engines) and information (stored in magnetic or optical signals and transmitted/processed electrically) is not at all due to chemical processes or links). As every kind of data storage and processing is prone to mistakes, there is a potential for evolution by mutation and selection.

Hence it can only be sustained beyond reproduction if losses remain acceptably low: for example, roots can sustain terrestrial plants only if they manage to sequester the essential elements by delivering just a part of the organic acids produced primarily by photosynthesis from CO_2, N oxoanions rather than all of it. The theoretical minimum here would be 3%: the above ratio $C/\Sigma_{metals} \approx 200$ combines with citrate containing six C atoms to the statement that 3% of assimilated C must be "invested" given there were no retrieval of C after resorption of citratometallates. If, however, plants have to use > 25% of their photosynthetic products just to mobilize metals from sediment (which implies most of citrate or malate is irreversibly lost to the soil as stoichiometry is constant) their chances to grow and reproduce at reasonable rates will shrink; anyway, they have no chance to "organize" a metabolism requiring larger metal amounts and smaller C/M ratios. Then, if there are alternatives like with hydrolases (Mg, Zn, Mn, Fe(II)) or in closing the N cycle by urease (Mn, Ni), the best metal "option" will just be efficient enough to make a modest living. Green plants and chemolithoautotrophs change compositions of the C, N precursors in a manner as to increase metal affinities (complex stabilities) considerably, and the same holds for transformations of common S, Se and sometimes P compounds taken from the(-ir) environs.

Obviously such N vs P selectivity depends on the ligand sensitivity x – more exactly x_{2d} – of the metal ion as shown in Eq. 2.32. Equation 2.32, and the above $E_L(L)$ differences (see Table 2.13) then yield a "necessary" $x_{2d} - 4$ to have P(-oxo)ligands prevail over N and −4 to −12.5 for C-oxospecies. Accordingly, among the essential elements which are rather abundant in biomass this does hold only for Ca and Mn while all others will be located at N- or S-binding sites. Other elements which should prefer P(-oxo) sites and thus interact with the "P switch" for proteins, include heavy alkaline earths Sr and Ba, REEs except for Sm, Tb and Eu (both oxidation states in the latter case) and tetravalent Ti, Zr and Hf (Table 2.3). Although P(O) activation is crucial to both energy metabolism and CO_2 fixation, hardly any of these metals is essential!

However, there is a role for the most abundant among these elements, Ca ($x_{2d} = -10.98$) in plants also which directs refers to P(-oxo) site interactions: by addition to certain parts of nucleic acids, Ca^{2+} causes uncoiling of DNA and thereafter translation and expression of certain parts of the genome. REEs are known to stimulate cell budding and growth of plants, which is exploited in agriculture in China and Canada, while Ti(IV) (budotitan and similar cytostatic agents (Fränzle and Markert 2003; Kopf-Maier 1994; Guo and Sadler 1999)) acts by modifying the P switch activation or desactivation of enzymes. Thus these ions with x_{2d} between −8 (Ba) and some −60 (Ti(IV), extrapolated from data for Ti(III)) all conform to the expectations from the above reasoning on partition to various sites. Of course, stoichiometry of metals with respect to N and P is also involved and influences the effect. While N/Ca ranges from 5 to 17, P/Ca ≈ 1, which implies that changes of "free" Ca concentrations can indeed control biochemical activities of phosphorylated

(or to-be-phosphorylated) biocompounds in an efficient manner (Sr and Mn (PEP carboxylase; Kai et al. 2003) might do so also in special conditions, the others are too rare as a rule).

Several of the investigated plant species, in particular, *Vaccinium myrtillus* and *Deschampsia flexuosa*, can accomplish direct uptake of organic nitrogen (Näsholm et al. 1998), absorbing (2-^{13}C- + ^{15}N-isotopically doubly labeled) glycine in unchanged state (≥91% in *Vaccinium myrtillus*), that is, without additional metabolic transformations in or around the rhizosphere. Given that glycine and other amino acids are efficient ligands released by drying mosses as well as by some grasses and mykorrhiza in the rhizosphere, this process will influence transport of metal ions into plants also, particularly of those for which stabilities of amino acid and hydroxicarboxylate complexes do strongly differ, like Cu, V(IV) and Ca. N uptake rates in organic states are similar to those of ammonium or nitrate ions for these plants including *P. sylvestris* (Näsholm et al. 1998; Persson et al. 2006) which means, at a ratio $N/M_{total} \approx 10$ in upper, green parts of the plant that this capability is significant in metal ion transport also, at least for Ni, Cu, V. Here, M_{total} excludes the (amount of) alkali metals which usually do not undergo complexation in the rhizosphere (but cp. the Cs-specific phenolate ligand which causes accumulation of caesium in the Bay Bolete mushroom [*Boletus badius*]). The four species involved have common to very low effective electrochemical ligand parameters in their photosynthetic organs (*Picea abies* −0.18 V in recent and −0.24 V for two-year-old needles, *V. myrtillus* −0.26 and *D.flexuosa* −0.165 V), similar to values for polycarboxylate ligands rather than amino acids. This suggests that, for a variety of only loosely related plant species which in addition are supported by quite unlike kinds of mykorrhiza, significant parts of transport and metal fractionation occur beyond the roots (where the doubly labeled ^{13}C- + ^{15}N-glycine was recovered (Näsholm et al. 1998)), with the root/shoot interfacial region being the most probable "bottleneck" or site of control.

Because analytic values for tree leaves (Schröder and Fränzle 1992; Pfennigsdorff et al. 1993) are similar and thus also close to the intersection point at −0.15 V (e.g. birch leaves about −0.19 V, pine needles −0.135 V (Fränzle et al. 2008)), feedback of trace metals including nutrients by leaf or needle litter is pretty efficient (Fränzle et al. 2008b) as there is minimum fractionation (data for feedback in such trees in Fränzle et al. (eds.) 2008) among the elements; thus their kinds of leaf litter feed back to soil more or less the same composition of trace metals which was taken up by the roots before. Obviously such a situation ecologically becomes most important if an ecosystem depends on close to 100% feedback/recycling of certain (metal-ion) materials, like in parts of the Amazonian hylea.

Ryegrass *Lolium perenne* differs from most other green plants in using (besides citric acid cycle intermediates) substantial amounts of amino acids – mainly proline and histidine – in root exsudate (Vernay et al. 2006). Given this, primary fractionation upon extraction of metal ions from soil solution should rather resemble that effected by fungal mycelia, especially if the imidazol moiety of histidine becomes involved in metal ion binding: histidine also is a principal component in xylem milk saps of (mainly nickel) hyperaccumulator plants (Farago 1986), suggesting imidazol is implicated in (sometimes tremendous) Ni enrichment; in fact, green parts of *L. perenne* also show a somewhat elevated Ni content of 3.0 mg/kg DM (Markert 1996). However, the calculation of $E_L(L)_{eff}$ for above-ground parts gives a value of −0.19 V for *L.perenne* which is below that for most understorey plants but closely similar to those of bidentate α-hydroxi- or ketocarboxylates (pyruvate, malate). As ryegrass does not require much N in the soil, recycling of N (amino acids, imidazol groups) "invested" in metal sequestering by oxidative des- or transamination in or just above the root zone is imminent. The C/N value for the leaves of *L.perenne* (no larger than 11) argues against complete oxidative ligand exchange and transfer to quite different carriers as observed in higher plants but apparently causes exclusion of rather weakly binding metals with yet positive x_{2d} for the direction of $E_L(L)$ change; accordingly its Cd content is rather low (0.12 mg/kg DM). Given the different values for c and x there is no contradiction to behavior of Ni in ryegrass: when histidine is desaminated, the product imidazolylpyruvate will use the N_2 ring system rather than the ketogroup in chelating some metal ion of sufficient (neutral N) affinity (distinctly positive x_{2d} value), including nickel. The data for various plant species are given below (Table 2.18): it provides an overview concerning the relationships among soil properties (redox potential, pH) and bioavailability of metal ions to plants determined by the electrochemical ligand parameter; columns 1–4 (left) are taken from Kabata-Pendias (2002).

There is a maximum in BCF values for metal ions having intermediate rather than "extreme" values of x_{2d} (Cd and Zn) which is against simple intuition.

Table 2.18 Relationships among soil properties (redox potential, pH) and bioavailability of metal ions to plants

Soil properties		Bioavailability		(How) does bioavailability depend on X_{2d}?	E_L (L), hapticity η of ligands present in soil
Redox state	pH	Well/easily accessible by plants	Moderately accessible by plants	(Qualitative (trend) statement)	
Oxidizing	<3	Cd, Zn, Co, Cu, Ni	Mn, V, Hg	Does increase with X	Distinctly positive
Oxidizing	>5	Cd, Zn	Mo, Sr, V, Se, Te	Best at X = 6 – 9; negative X and oxoanions cause lower BCF	η ≥ 3 (stepwise deprotonation of multidentate multi-acids?)
Oxidizing, rich in Fe	>5	No metals well accessible/ displaying high BCF	Cd, Zn	Strong adsorption yet shallow BCF maximum at X_{2d} = 6–9	η ≥ 3 (stepwise deprotonation of multidentate multi-acids?)
Reducing	>5	Se, Mo	Cd, Zn, Cu, Mn, Pb, Sr	No clear trend	Oxo-/thionions (Se, Mo) favoured over M^{2+}
Reducing, free H_2S Present	>5	No metals well accessible	Mn, Sr	Thioanions also retained in soil except for poor sulfide formers (neg. X_{2d})	Sulfide precipitation limits transfer into plants

Figure 2.16 gives an account for this: BCF values for different metal ions are determined by a competitive equilibrium among root (mycel) exsudates and soil-borne ligands for the metal ions, in addition there are various ligands (many of which mutually interconvert with time and soil aging). This causes the BCF maxima observed with Zn or Cd (or Ni) in oxidizing (neither Zn nor Cd nor Ni undergo redox speciation in soils, this is just an effect of the ligands!), neutral or but very weakly acidic soils. Fractionation and hyperaccumulation tendencies can be extracted from the nomograph in Fig. 2.16 also. One must distinguish between N-containing and N-free (O-donor sites mainly, e.g. carboxylate or phenolate) ligands here:

- The reduced C/N ratio which denotes the ratio of amounts of entire ligand molecules (rather than of the specific atom kinds) of N-containing (amino acids, peptides, chitin, hydroxamates (bacterial and fungal siderophores), certain phospholipids (with cholin, sphingosin or amino acid side chains)) vs N-free ligands (oxalate, citrate, malate, oligophenols, other humic materials, glycolipids, etc.) in soil solution/solid fraction, and
- The difference between electrochemical ligand parameters of the locally prominent representatives of both kinds

Equations 2.4 and 2.6 imply complex stability in binding a certain metal ion to increase with changing electrochemical ligand parameter in an exponential manner, the **lowest** cumulated complex stability and thus retention by the soil result when complexes formed by the N-containing and N-free soil ligand fraction are equally stable. In case of any difference, there will be exponential and thus more than linear increase of one of the two stability values, letting the sum increase likewise. The result of minimum retention by soil is maximum BCF (cp. Tyler 2004b). The pink area in the lowermost left part of this nomogram refers to the heavy alkaline earths (hyperaccumulation of Ba is seen in paranut trees, for example, Emsley 2001) and to the REE ions except for Sm, Eu and Tb which are distinguished by slightly to distinctly positive x_{2d} in their trivalent states also (cp. Fränzle et al. 2008a).

As anticipated, some non-essential elements may respond to biological redox gradients in a quite similar manner as Fe does because there need not be any direct transformation during photosynthesis: **europium** which both undergoes ready, high-quantum yield photoreductions when irradiated (λ ≥ 340 nm) together with alcohols, sugars or amino acids (Horvath and Stevenson 1992, see below), and the $M^{(II)/(III)}$ redox potential of which is not too low either, will become separated in distribution from all the other REEs (La, …, Nd, Sm, Gd, …, Lu) including yttrium in those 13 species investigated by Markert (1996), and likewise in other plants. Reductive magnetite formation and that of Eu^{2+} being related is the more conspicuous as both trivalent forms

similarly strongly interact with citrate (the correlation coefficient of Fe/Eu abundances is +0.905 for the 13 plant species).

Europium might be an efficient tracer of certain leaf/needle-related processes exactly for its differences to other REE metals which give rise to both an "uncommon" c/x value couple (inside the "window of essentiality") and fractionation both vs other REEs, Al in plants (Markert 1996) and in the environment, e.g. the ocean (Nozaki 1997). After combining with alcoholes or biogenic ligands such as sugars, sugar acids, oxalate or amino acids, Eu^{3+} readily undergoes photochemical reduction at $\lambda \geq 300$ nm, often even in visible light (Horvath and Stevenson 1992). Here, $(Eu^{3+})^*$ is quenched by excited porphyrines like chlorophyll by electron transfer; moreover, this ion is readily reduced by thermochemical processes, with the corresponding potential (−0.35 V vs NHE) being well within the range accessible in biochemical reductions (redox potential data and extreme products of the latter reductions are listed in Thayer 1995). So it can be anticipated that in photosynthetic organs (to which Markert's set of data refers) europium will undergo fractionation from the other REEs and from Y to get enriched/distributed in a manner rather similar to that of (other) divalent ions of rather feeble ligandophily, that is, similar to alkaline earth dications (including MSO_4 precipitation (Donohue 1977)). In fact, whereas abundance correlation coefficients are very close to +1 among the REEs usually, which also holds for Y, and negative towards alkaline earths, the corresponding r values for Eu differ strongly (Table **2.20**, after Markert (1996)); ytterbium is listed because it might undergo the same reaction but at shorter wavelengths or far more negative potentials while Gd is considered a "colloquial" REE ion and data for Y, Al are given for comparison.

While abundance correlations of Eu with Ba and Sr, although far higher than for other REEs (i.e., positive), must be considered insignificant, the effect becomes pronounced for those (less heavy) alkaline earth metals which are not only essential but also form more stable complexes, that is, Ca and in particular, Mg. Al goes along most of the REEs except for Dy and Lu. Accordingly, Eu(II) complexes with biogenic ligands are to be expected in leaves or needles, perhaps these then may be – like those of (then trivalent) other REEs – discernable by fluorescence.

Since photochemical re-oxidation of Ce(III) is not feasible in biological material at wavelengths which make their way to the ground (yet; $\lambda \geq 295$ nm), and biochemical oxidants capable of effecting redox potentials far higher than +1.2 V vs NHE are rare, no corresponding deviation from general REE distribution patterns is observed with cerium (Table **2.19**).

There are no peculiarities with Ce, unlike with Eu, suggesting that the oxidation state Ce(III) does not change within (these) plants, in no phase/step of transport. Likewise, it would not in either La or Pr.

So, there are no differences between Ce and either La or Pr with respect to distribution among various biological materials, strongly suggesting there is – and remains throughout all the steps of biochemical sequestration, transport, etc. – Ce(III) only, not the tetravalent ion, in all these plant species. Except for the fractionation of Dy and Lu vs Al, the distribution of Lu being not at all correlated with that of Al, there are no other hints of any REE (including Y) to be fractionated vs any standard in a way suggesting (photo-)biochemical redox processes to be involved. Strictly speaking, Lu^{3+} is no member of the REE series as f orbitals are no longer involved in either electron acceptor coordination chemistry or any redox process which might (but does not) occur in condensed matter with lutetium. Thus, the "deviant" behaviour of lutetium might be anticipated. As a result, Eu does go along with alkaline earths rather than Y, REEs (Table 2.20).

Europium behaves differently, presumably owing to photochemical reduction which makes it be enriched or deposited rather together with alkaline earths, not Ln^{3+} species. Note the large differences between Eu and its two direct neighbour elements Sm and Gd. Correlation coefficients for abundances among different plant species are taken from Markert (1996).

In a "real" environment – other than hydroponic nutrient solutions – it is most unlikely that the differently large demands of all the essential elements are

Table 2.19 A comparison of abundance correlation coefficients of the three lightest REEs La, Ce and Pr vs other (usually) tri- or tetravalent ions

metal	La	Ce	Pr
Y	0.964	0.973	0.967
Fe	0.932	0.924	0.88
Cr	0.42	0.44	0.44
Eu	0.86	0.82	0.89
Al	0.84	0.82	0.82
Ti	0.78	0.79	0.79
V	0.92	0.92	0.91

Table 2.20 Correlation coefficients for abundances of several elements (alkaline earths, Eu) vs those of certain REEs and Al in 13 plant species (from Markert 1996)

Metal	Ba	Ca	Mg	Sr	Eu
Y	–0.11	–0.23	–0.40	–0.08	+0.82
La	+0.17	+0.115	+0.46	–0.02	+0.86
Ce	+0.16	+0.115	+0.39	–0.02	+0.82
Sm	+0.14	–0.16	+0.04	–0.04	+0.905
Eu	+0.23	+0.35	+0.74	+0.15	1.00
Gd	–0.12	–0.25	–0.40	–0.09	+0.85
Yb	+0.115	–0.24	–0.40	–0.09	+0.84
Al	+0.03	+0.19	+0.60	–0.01	+0.86

precisely matched at one given time and point of its lifecycle in any species which is exposed. As pointed out before, anabolic activities and those aiming on reproduction increase the minimum AC orders which are necessary, albeit to different extents depending on the manners of reproduction. To give an example, the tricarboxylate cycle which involves catalysis by Fe, Mg and Mn by itself is a critical one (Clarke 1975, 1980), and, provided there are sources of NH_4^+ ions and electrons, is coupled to amino acid biosynthesis affording either glycine (in plants, with the precursor glyoxylate derived from malate cleavage (→ glyoxylate + acetate)) or glutamate (in animals) while either amino acid – besides the Krebs cycle intermediates citrate and malate – is delivered by many plants (in particular, grasses) to mobilize and sequester heavy metals from soil. As a result, there is direct autocatalysis: Fe, Mg and Mn interact to produce species which can be used to obtain these very elements from soil, and then are used to produce the sequestrants again. The AC order in this case is fairly high even though some larger part of the sequestrants gets lost to the soil liquid and can be detected there at levels up to several mmol/kg of soil. However it is hard to quantify these amounts by means of, e.g. COD determinations using aqueous soil extracts (Kollaske 2009, *Diploma* thesis). This condition is by no way restricted to any single element but holds equally for all the elements required by the corresponding species. If just one is missing or lacking severely, growth and reproduction are stopped altogether, giving proof of the necessity of all these elements by controlling growth and reproduction which re-commence after administration of the hitherto lacking element (exactly the definition of essentiality given by Arnon and Stout (1939)). Even much earlier Liebig (around 1850) referred to this as the "principle of minimum": the "least available" (essential) chemical element will limit growth and reproduction. This can be readily translated into SNA terms: if there is **one** critical or even weak cycle interwoven with all the other (critical or arbitrarily strong) ones, the entire collective of cycles which are strongly and multiply coupled in biochemistry will be thoroughly decreased in its autocatalytic efficiency, to an extent that it probably will no longer act in an autocatalytic manner, precluding both cell-budding – required for growth – and reproduction. The corresponding cycle can be dubbed weak or sub-critical, yet this situation cannot be sustained over any substantial part of the organism's lifetime, whether it is due to lack of administration/take-up or to increased consumption by some parasite – directly or indirectly.

So, given the considerable demands for Mn in water photooxidation (average Mn levels in leaves or needles of terrestrial plants are 250–700 μg/g), with PS II manganese susceptible to chemical perturbations such as formation of nitrosyl or nitrocomplexes if exposed to NO or NO_2 (Ioannidis et al. 2000) (the N oxides may also react with tyrosyl radical intermediates there), plants must find a way to yet retrieve it from aerated sediments into their organisms. Relocation to extracellular enzymes like in ligninolytic fungi is not an option in (terrestrial) green plants as photooxidation of water to obtain electrons for CO_2 reduction requires light and thus must occur above the soil surface. Plants can overcome this problem due to the existence of an intermediate oxidation state, trivalent Mn, because Mn(III) forms very stable oxalatocomplexes (Mizerski 1997; Scheffer et al. 1998) which in turn can be resorbed and reduced inside the roots: otherwise Mn might even be lost from the root system backward to an oxidizing soil environment.

The situation is even more difficult in marine biota owing to a concentration of dissolved Mn of but 0.4 nmol/L (Nozaki 1997). In marine conditions Mn oxide precipitation also occurs albeit in depths where photosynthesis is impossible for lack of any light (Mn nodules occur between 4,600 and 5,300 m depth). So, by air oxidation Mn may become inaccessible to organisms which use it in photosystem II, pyruvate carboxylase, arginase, malate enzyme (Vallee and Williams 1968; Höhne 1980; Kaim and Schwederski 1993) while reduction neither is a straightforward option (due to the fact that Mn(II) complexes are fairly weak) but there is a compensating, if not even Mn-"pumping" mechanism: Mn supply of marine phytoplankton is augmented by photooxidative dissolution of colloidal

Mn oxides reacting with DOM or/and Br^- ions (Sunda and Huntsman 1988).

For the history of Mn supply in a changing ecosystem (ongoing eutrophication in a lake, formation of a B horizon and possibly sandstone, iron pan layers in some recently deposited terrestrial sediment) one must consider temporal trends in the electrochemical ligand parameters of geo- or limnochemically important organic compounds (SOM, DOM): whereas it rises in soils (Fränzle et al. 2007), it will be decreased in standing waters due to partial photooxidation, including N- and S-containing DOM fractions and including even synthetic aminopolycarboxylates (Fränzle et al. 2005):

- By (often photoinduced) oxidative deamination of amino acids ($E_L(L) = -0.05$ V) coordinated to Fe(III), these are converted to 2-oxocarboxylates or glyoxylate and eventually oxalate ($E_L(L) = -0.17$ V)
- Thioethers ($E_L(L) = +0.35$ V) like methionine form thiolates ($E_L(L) \approx -0.54$ V) by CS-bond cleavage and finally COS, SH^- and sulfate
- Polycyclic phenolic humic substances produce phenol carboxylates (oxidation of adjacent rings) of the salicylate type and possibly 3-ketoenolates ($E_L(L) = -0.08$ V for both $acac^-$ and $sal2^-$)

During this aquatic photooxidation, Cu(II), Al(III), UO_2^{2+} or VO^{2+} become considerably less tightly bound to DOM and thus get more bioavailable (which, however, spells "more toxic" when these ions are no longer kept from attacking fish gills by DOM complexation (Paquin et al. 2003)) while Mn(II), Mn(III), Ca, Sr, Ba or REEs except of Sm, Eu and Tb now readily coordinate and can be resorbed as small (almost) neutral species. Since x_{2d} is negative for Mn^{2+}, carboxylato- and oxalatocomplexes of Mn(II) are more stable than the phenolato- or amino acid complexes, and MnS is readily soluble also (Mizerski 1997). Abiotic photooxidation using biogenic organics (DOM) thus becomes a supporting factor for uptake of Mn into PS II of plants and other biochemical sites. Obviously, this means that

- A certain – not too high, neither too low – share of cell biomass must be converted into DOM by release or cell destruction (other than feeding).
- The C/Mn ratio and the yield of assimilated C per mol unit of Mn used in PS II must be sufficiently high. In terrestrial plants, C/Mn = 600–2,000 by mass and about 2,800–9,000 in molar ratios.

While many plant parasites directly take up products of photosynthesis or compromise element uptake by eating parts of the roots or extracting, e.g. phloem or xylem saps, there also can be indirect effects: plants respond to attack to certain parasitic insects (gall wasps) by producing novel structures in order to contain and confine their parasites (Fig. 2.17), thereby producing both stress signals and communicating to nearby plants (also those belonging to other species) and trying to poison the parasites by producing additional polyphenols (gallic acid and others), especially in oaks. Apart from the fact that gall wasp larvae have evolved to the point where they can cope with these polyphenols by metabolizing them (much like earthworms do), the latter polyphenols will scavenge Fe(III)ions (iron-gallic ink!), compromising plant biochemistry around the galls and causing typical chloroses there (Fig. 2.17). So the defense against gall wasp parasitism is not really effective in toxicological terms and moreover influences the plant's own biochemistry by trapping iron (and Al).

Total stoichiometry of the processes involving a given metal ion as biocatalyst which puts an upper limit to its O_{ac} (Eq. 2.24) may vary widely: from 3 or 4 up to dozens or even hundreds with elements like zinc, copper, magnesium or iron. If the complete biochemistry of some organism is formulated as a set of reaction equations including the respective (bio-)catalysts and their possible central ions, Zn, Fe, Mg, Cu will thus have AC reaction orders of ≈100 in higher organisms including higher (vascular) plants, whereas Mo, Ni or V do catalyze just a couple of transformations, giving rise to a reaction order of about five (Only some 30–40% of proteins contain metal ions essential for accomplishing their functions (Höhne 1980; Williams 1983)). The latter cycles thus are more sensitive towards perturbations, possibly causing some of these elements to "vanish" from the set of essentials (cp. Frausto Da Silva and Williams 2001 for biochemical roles of Co and Ni) eventually; in addition, such perturbations are more likely to influence such a sensitive cycle. An example of this behavior may be Mo because

(a) Mo is involved in many redox transformations, including those which give access for a plant to elements as crucial like N and S.
(b) There are chemical antagonists to Mo uptake, mainly Cu.

Interestingly enough, hyperaccumulation in plants most often involves Ni (the main use of Ni in higher plants is associated with urease and thus with nitrogen cycling and retention), rather frequently also Al or REEs which are not at all essential while "strongly

autocatalytic" bioelements like Cu, Zn are much less often involved. There is one geobiochemical reason for this (see Table 2.18), namely the link between plant uptake/retention by soil ligands of certain elements depending on their x_{2d} values. Apparently hyperaccumulation is not at all related to any biochemical role of the corresponding element, yet, besides Ni, two other, more frequently essential elements, namely Mg and Zn have BCF values ≥1 in "ordinary" vascular plants. For all three elements, x_{2d} is almost identical. Given any one of these partial AC cycles gets weak, it precludes the entire system of which it is some part from strong or critical autocatalysis: if the organism does survive this situation at all, it will neither grow nor reproduce as long as this depletion situation carries on. This is a reason for Liebig's principle of minimum and the basis for effective determination of essentiality by exclusion of certain elements. Section 2.2.13 will provide an example for this, the cycle of Mg in plants, which is prominent under aspects of both photosynthesis and the amounts of metal involved.

As noted above, the principal ratio among essential trace metals in **animal** physiology will be that between Mo and Mg. With energy-releasing metabolism in animals prevailing over the anabolic one, this Mo/Mg ratio – which in animals like in plants, common soils (Kabata-Pendias and Pendias 1984) or freshwater (Markert 1994) is about 10^{-4} in many cases (stoichiometric ratio (Table 2.16)) – should be kept constant, which, introducing the c- and x-values of Mo(VI) and Mg, means $E_L(L)_{eff}$ = +0.15 V and $-\log k_{diss}$ = 5.2. In terrestrial plants, Mo contents do vary by at least two orders of magnitude whereas [Mg] is constant within some factor of 10 (Markert 1996 and data in this work). Thus Mg/Mo is subject to considerable variation in terrestrial plants also, and their consumers can "run" their digestive tracts with less exotic, i.e. somewhat negative $E_L(L)$. In addition, reducing conditions in digestive tracts will influence Mo uptake by its reduction. Now compare this to the ambient Mo and Mg concentrations which are of order 1 mmol/L (Mg) and 100 nmol/L (Mo) in freshwater. The actual effective electrochemical ligand parameters for zooplankton organisms are far lower than $E_L(L)_{eff}$ = +0.15 V but even if this value would be reached, the outcome would be a massive loss of some other essential metals vs both Mo, Mg, which indeed is observed in some limnetic consumers. This value differs strongly from the intersection point of the lines at some −0.15 V which is next to $E_L(L)_{eff}$ for many terrestrial plants in moderate climates (but neither for limnetic ones (≥−0.1 V) nor for such (<−0.26 V) in the semi-arid tropics (Mole River National Park, Ghana). Accordingly, there would be substantial fractionation in a terrestrial plant of such properties, and, with the $E_L(L)_{eff}$ value being far larger than the minimum of some −0.2 V, there would also be enrichment according to the Irving–Williams series, thus favoring Cu (and Ni) over Zn, Mg or Mn. The actual value, however, is lower than this, with an inverse fractionation and corresponding difficulties in Cu, Ni retention.

There are obvious consequences for metabolism of these consumer animals feeding on limnetic algae as they – and likewise the fishes which feed on them – are to maintain a corresponding internal metal concentration. This can be expressed in a flux diagram which becomes most simple for marine food chains as concentrations of dissolved Mn, Fe, Cu or Ni all are <1 nmol/L there while $[Zn^{2+}] \approx 6$ nmol/L and $[Co^{2+}] \approx 20$ pmol/L (Nozaki 1997) at appreciable DOM (and thus organic ligand) concentrations. DOM complexation further aggravates the problem of retention of whatever metal content was taken up from food inside fishes as it promotes loss to the ambient water. In terms of ecological stoichiometry, the extent of loss may be calculated as follows: the oxygen concentration in water is some 100–350 μM/L, which in aerobic metabolism enables oxidation of some 10^{-4} mol C_{food}/L oxygenated water perfusing the gill system. Upon biooxidation of (algal or other) food by consumers, metal ions will be liberated to an extent given by C/metal ratios in the food. In marine biota there are (see above) considerably higher concentrations of the above metals, unlike for Mg, V, Mo in algae than in the aqueous surroundings of a marine crustacean (e.g. a gammarid) or of a zooplankton-consuming fish like a herring (*Clupea harengus*). Hence the complexation properties of their respective biomass must be such that they can retain 3d ions against "pull" by DOM (which, for example, binds >90% of total Cu in fresh and ocean waters) and braving a concentration gradient, that is, $-\log k_{diss[animal\ biomass]} >> -\log k_{diss[DOM]}$. The latter $E_L(L)_{eff}$ value for DOM can be obtained from fractionation/speciation of freshwater metal ions into DOM and is about −0.15 V once again.

As for the above metal ions other than Mn^{2+} $x_{2d} > 0$, $E_L(L)_{eff}$ for biomass of marine plankton consumers must be higher than this value. Concerning zooplankton, this must be regarded as an average value once again as chitin forms a substantial part of their biomass

(i.e., the exoskeleton) and is directly exposed to both water (outside, using in biomonitoring) and haemolymphe (inside, there are no blood vessels in arthropods); from metal speciation next to chitin $E_L(L) = -0.28$ V was calculated using Eq. 2.10 which means that the values for both arthropod inner organs and the haemolymphe freely flowing around them outward to the chitin exoskeleton must be considerably higher.

Chemical thermodynamics, i.e. ambient redox potential, apparently controls C/N or the amount of nitrogen which can be allocated to/retained in some organism. Now redox chemistry which might require certain oxidants is also involved in making the ligands which sequester the metal ions from soil. Therefore, if ligands are used which contain nitrogen themselves but must operate at low ambient redox potential (very wet soils, for example), it might well turn out that their own average N content exceeds that of the entire organism they are meant to supply with trace metals. If so, the above organism can only survive if most of the ligand and thus of its nitrogen is actually retrieved. This problem is most pronounced in aerobic fungi while green plants which spend some N (in amino acids) to obtain metals, do so usually in well-aerated or at least nitrate-containing soils. Further apart from green plants in terms of taxonomy, this problem also exists with Fe(III) reducers which have C/N > 100 but employ cyclic oligopeptides and sometimes also hydroxamates to mobilize Fe(III) from solid oxides. The limits which then are upper limits for C/N ratios are given for a number of different organisms (Table 2.21):

2.2.13 A Comprehensive Analysis of Autocatalytic Processes Within Some Photosynthetic Plant

Relationships between (essential) metal ions and the biorelevant ligands (apoproteins, porphyrins and other N-containing polycyclic compounds, (deprotonated) peptides, organophosphates, etc.) are two-sided in

Table 2.21 Sequestering ligands and minimal (external, ambient) redox potentials required to produce them from ambient reactants still present in soil or groundwater

Sequestering ligands	Required educt/ organic precursor	Additional reactants for synthesis of ligand	Minimum value of $\varepsilon_{pH} = 7$ (V)	Log C/N ≤	C/N ≤	Real values of C/N
Hydroxioligo-carboxylates (citrate, malate)	Acetate, oxalate	No special, unless there is CO_2 reduction	−0.12	2.055	113	Plant roots (not woodified): 10–40
Oxalate	–					
Hydroxamates	Ketone enolates	NO	0.22 (nitrate as NO precursor)	1.410	26	7 (soil bacteria); 10–15 (fungal mycelia)
N-hydroxy-bis-carboxylates, e.g. amavadine	Tertiary amines or tert. amino acids, forming N-oxides to undergo olefin elimination	Dioxygen or H_2O_2 [Fe(III)]	0.33 (dioxygen)	1.200	16	About 12 (*Amanita* spp.)
1,2-diphenol(ate)s		Dioxygen or H_2O_2; $\{Cu^{2+}\}$	0.33 (dioxygen)	1.200	16	<10 (Fe(III)-reducing bacteria)
Amino acid(ate)s		$RCO\text{-}COO^- + NH_4^+$, pyridoxal phosphate	About −0.1 (glycollate/ glyoxylate redox couple pertinent to plants)	2.01	102	ca. 30 (drying mosses, grasses like *Lolium perenne*)
Phosphorylated sugars, e.g. inositol hexaphosphate						

For all these cases, real C/N values of organs or interfaces in contact with soil are far within the theoretical limits

general and also in green plants; there is control of the metal ion by its ligand mixture environment while other parts of biomass and products of metabolism bring about uptake of this very metal ion. This interplay organizes resource tapping as well as loss processes which also can be described in quantitative terms. To give an example of "metal ion-non-use", on the other hand, it is now established (Zabinski and Toney 2001) that coordination of pyridoxal phosphate (PLP) to metal ions is generally obstructive to processes such as hydride transfer (oxidative transamination) or decarboxylation promoted by PLP even in those cases where there is pronounced complexation with oxophilic Lewis acids like Al(III), Y(III) which, by polarization and partial charge transfer, could be expected to support these transformations (general electrophilic catalysis). The catalytic effects observed in vitro (Metzler et al. 1954, Ikawa and Snell 1954, Krause 1959)) are simply due to a faster formation of the benzylidenamine (Schiff base) from PLP and amines or amino acids. The further transformation rather is inhibited. Since oxidative desamination of alanin(-at)e effected by PLP is much (75 times) faster than that of ethyl amine, and aminomalonate is more rapidly decarboxylated than glycinate, PLP reactions abstracting CO_2 or one pair of electrons apparently proceed faster with higher electron densities at site, thus any complexation of PLP phosphate (or phenolate) sites by some M^{3+} should reduce catalytic turnover rates substantially. The methyl group apparently avoids chelation for steric reasons, making PLP less sensitive towards metal ion effects than the methyl-free pyridinium salt. So it comes to no surprise that PLP-using oxidoreductases or decarboxylases do not employ any metal ions. REE ions such as Y which are most liable to organophosphate coordination, thereby enhancing rates of polyphosphate (e.g. NTP) hydrolysis, accordingly block PLP activity altogether, as they do with ATPase and glucose-6-phosphate dehydrogenase (Deuber and Heim 1991).

There are some multifunctional "biometals" like Mg and Mn(II) the complexes of which are rather labile in both kinetic and thermodynamic terms (Taube 1952; Riedel 2004; Jordan 1994), in spite of their high AC orders. This situation goes beyond the description by Sabatier's principle: every (bio-)autocatalytic process promoted by such metal ions is going to have an exit order near 1 at least, summing up to an exit order >> 1 for such metals. This also holds for photosynthesis: the lifetime of chlorophyll in organic or aqueous-organic solutions, even such saturated with Mg^{2+}, under direct solar illumination is just a few minutes, precluding its use in photovoltaic or photoelectrochemical devices. PS II is more stable and can be coupled to the famous $[Ru(bipy)_3]^{2+}$ photocatalyst, giving rise to more efficient water splitting molecular devices.

Notably chaperons which might be thought to allocate metals ions like these back to their biological sites rather control distributions of strongly binding metals like Cu, Ni; thus the heavy alkaline earth metals, Ti or REEs do not display any functions in biocatalysis: apparently chaperons will not accomplish this, can withdraw or withhold rather strongly binding metals like Cu, Ni, Zn, Cd, Al, also some non-metals such as As (Tottey et al. 2005) but are unable to allocate weakly binding ions to some metalloprotein. Rather, weak complexation is used for chemical signal transmission, even in cases where values of c and x preclude multiple modes of biocatalysis: Ca^{2+} has its prominent role in signaling and also changes molecule shapes of enzymes, opening or closing the catalytic cleft and thus controlling their activity. In cell division, concentration density waves of Ca^{2+} which determine budding in an autocatalytic manner are unleashed on the cell surface: Ca release causes release of more Ca^{2+} from storage species and thus induces a chemical wavefront much like oxidation reactions promoted by protons do (cp. Pota and Stedman 1994).

To give a real example, have a closer look on main functions and cycle of **magnesium in green plants**. Control on autocatalysis depends on the principal functions of Mg, that is, on photosynthesis: when substantial parts of Mg taken up by roots are allocated to chlorophyll and rubisco synthesis, less will be available for other metabolic pathways, reducing the turnovers there unless there are lots of Mg around like in marine plants. In addition, the tricarboxylate cycle (citrate cycle) requires Mg (besides Fe and Mn) to produce the enzymes; hence some Mg (as well as Fe, Mn) must be "invested" to produce the citrate (malate, oxaloacetate (aspartate)) ions delivered by the roots to render Mg (and other metals) in turn bioavailable by means of complexation and resorption of almost neutral complex entities. Furthermore, the tricarboxylate cycle is coupled to biosynthesis of amino acids by redox transamination; hence there will be both competition at the metal center(s) and possible extraction of metal ions from enzymes once NH_3 and electrons are

available for transamination towards 2-oxo-carboxylates (glyoxylate/glycine couple in plants (Heß 1999), 2-ketoglutarate/glutamic acid in animals). Glyoxylate is not directly obtained from the tricarboxylate cycle but both glycinate and glutamate act as two-dentate ligands (Sovago et al. 1993) and thus compete in biofluids with tricarboxylate cycle intermediates for the biocatalyst metal ions. Although citrate is a tridentate ligand, glycinatocomplexes of both Mg and Fe(II) are more stable (Mg: $-\log k_{diss} = 3.45$ [glyc] vs 2.8 [cit^{3-}], Fe(II): 4.3 [glyc] vs 3.2 (Furia 1972; Sovago et al. 1993)). For Ni(II), which does not take part in the Krebs cycle, dissociation constants of both citrato- and glycinato complexes are similar. When numbers of C- and H-atoms in ligands formed in the tricarboxylate cycle decrease after acetylCoA abstraction (to $n_C < 5$), complex stabilities towards Mg or Fe(II) are decreased considerably, also (e.g. malate/Mg: $-\log k_{diss} = 1.55$).

The way how **two** external (side-)loops regulate Mg-based autocatalysis in green plants, namely

1. Amino acid synthesis from citrate cycle products which indirectly extract Mg from the citrate cycle because the corresponding complexes are more stable than those with citrate, malate …, reducing efficiency of reservoir tapping via the roots, and
2. Photosynthesis which directly and doubly uses Mg (see below)

The complete feedback pattern is depicted in Fig. 2.18.

In a network of autocatalytic feedback loops (of which we here do consider only those processes related to Mg) control functions which give rise to either autocatalyst supply (sequestration from the environment) or loss (abstraction or precipitation by "parasitic" byproducts) several different chemical species may interact or cooperate to achieve the above kinds of control (Eiswirth et al. 1991b), two such side-loops cause somewhat increased topological network complexity rather than fundamentally changing network dynamics.

The tricarboxylate cycle is an ideal example as it

(a) Both occurs in (almost) all living beings.
(b) All its steps, intermediates and catalyzing enzymes, including metal ion catalysts in the enzyme centers, are completely known.

The *stoichiometric* ratio C:Mg (not the mass ratio) is about 1,000 in the leaves of typical higher plants (recalculated from Markert 1996, 1998) which also holds for several kinds of marine macroalgae (Fränzle et al. 2008, unpublished) while the number of Mg-dependent enzymes and thus functions is ≥50 in a given species (Williams and Frausto Da Silva 1996). Mg transport and allocation are not controlled by chaperons anywhere, unlike elements like Co, Cu, Zn or "toxicants" such as As, Cd or Pb (Tottey et al. 2005). C_{2d} and x_{2d} values for Mg^{2+} are such as to predict formation of stable complexes (c = +3.94; x = +8.24), with rather high concentrations present in serum. These facts combined, it is expected that "occupation" of binding positions by Mg^{2+} occurs rather close to equilibrium conditions which implies that citrate cycle intermediates compete with apoproteins for Mg^{2+} the binding of which is kinetically labile (Jordan 1994).

This equilibrium assumption radically simplifies analysis of data, starting consideration with the above C/Mg ratio. There is but some part of organic C relevant here, namely, oligocarboxylic acids existing freely in serum in appreciable amounts. As shown in Table 2.17, the N/Mg ratio is considerably smaller – often <50 – whereas amino acid complexes of Mg are not much more stable than malato- or even citratocomplexes (Irving and Williams 1953; Furia 1972; Sovago et al. 1993; also consider Fig. 2.9 of this work). Hence, as N/Mg is so much smaller than C/Mg, one can neglect N-donor ligands entirely for understanding the competition between simple anions and apoproteins (to become Mg-based enzymes) for Mg, including contribution by the very products of the Mg enzymes (and this also holds for ligands containing S, Se, etc.).

Photosynthesis adds another feature, which can hardly be called a side-loop anymore, to Mg functions and cycling in plants which contributes to autocatalysis by product expenditure as shown in Fig. 2.18 above.

Chlorophyll and ribulosebisphosphatecarboxylase/-oxidase contain and require Mg^{2+} ions. Some smaller or larger part of the Mg budget of a plant, depending on light intensity and CO_2 levels in atmosphere or ambient water (there are differing CO_2 partial pressure demands in C_3 and C_4 plants, the latter using PEP carboxylase), is purposely diverted from construction of Mg-dependent proteins (other than rubisco which is the largest share) and thus from the "classical" AC cycles referring to Mg. PEPC levels also respond to Mg concentrations in local soils (Bardgett 2005; Kai et al. 2003). Of course, photosynthesis will contribute to maintainence of these cycles sooner or later also by autotrophy (this is why the term "investion", rather

than "expenditure", was selected above to describe this situation) but secretion of citrate or malate for Mg recovery happens at quite another site of a plant (in and around (mykorrhiza) the roots) than photosynthesis (Figs. 2.6 and 2.13). The fact that different functions in Mg sequestration and uses are distributed well across the organism of some plant renders the strong cycle associated with Mg even more tractable (if not "really" stable) than otherwise because the distribution of Mg functions in space and time will rather curb autonomous chemical oscillations or chemical wavefronts (Clarke 1980; Pota and Stedman 1994) as it demands for transport of Mg among the sites. The strong cycle is spatially reorganized into some highly interwoven, thoroughly connected network of AC subcycles each of which depends on products of the others but without abandoning spatial separation of events and (autocatalyzed) functions (in metazoans at least). If the system would be closed concerning matter (batch reaction conditions) which is most unphysiological, AC orders of Mg, C (and the other directly involved elements such as Fe, Mn (PS II, tricarboxylate cycle) or N) would become identical by every subsystem consuming the products of the others and in turn supplying them: that is, the situation described by Eigen's hypercycle model. A real plant, however, absorbs C from the air, to lose some of it into soil (root exudates, leaf litter (Farrar et al. 2003)) while the gross way of N, Mg (and the other metals) is the other way round, i.e. upward: absorption by the roots, loss (by littering) at the top (and for metals, also into wood which contains but traces of N when no longer taking part in metabolism). This antidirectional movement of C vs the rarer elements will cause the C/element ratios (which we hitherto considered for photosynthetic organs mainly) to vary depending on the position inside a plant. Sinks of different elements – even inside an organism, are located at different sites, with the local AC orders and thus cycle characters depending on the number of element functions at this very site: Mo is most important in roots (and probably strong Mo cycles confined to these parts of a plant) while Mn (activities, of both PS II and Mn enzymes in mitochondria) prevail(s) in photosynthetic organs; in addition, there is decoupling of the weakly bound and thus hard-to-retain manganese from equilibration with sinks, e.g. constructed by Mn oxide precipitation (even though attack of gases like NO_x on PS II manganese sites creates problems also).

This effect can be seen from Table 2.15, depicting distribution of some elements within Scots pine; for most metals other than Mn, levels in bark are highest except for those in the needles and tiny roots. Apparently this is how the hydroconductive system manages to bypass metals – including those which form anions, rather weak complexes while not being required by plants at all, like vanadium – along the internal sink of wood. For **nitrogen**, the situation is in between these extremes of stoichiometric ratios either increasing or decreasing steadily upward in some vascular plant: cellulose, lignin, and thus wood and lipids except for lecithin, sphingosins are devoid of it but everywhere where there are proteins, where there is biocatalysis, there is appreciable N (1–2% on average). By its role in nucleic acids, it additionally "communicates" the results and gives blueprints for autocatalysis concerning many other elements. To give an average value, 7.5% of assimilated C (that is, 2.5 times the theoretical value based on citrate) make their way into root exudation (Farrar et al. 2003), with another 6% (rather more in P-limited soils) being transferred to mykorrhiza and the same amount to soil microbes. In the end, 1.5% reside as root leachates while another 1.8% produce soil microbe biomass and 4.2% are respirated to reproduce CO_2 which mostly leaves the soil. This balance allows for refining the above estimates:

1. Much less C from assimilation is left behind in the soil for retrieval of metal ions by back-resorption not (yet) accomplished than goes into soil bacteria and mykorrhiza both of which will degrade the complexes and release (or absorb themselves) the metal ions. The total efficiency, allowing for reconsumption of citrate, malate, oxalate, amino acids thus used before in the roots thus is about 20% at best; ≥ 80% are lost to soil or other local organisms at least in the short time. Yet, there may be almost total (perfect) recycling linked to litter degradation like in Amazonia, allowing for closed metal cycles, too. For avoiding fractionation, the material (both degraded litter and soil (-forming) organisms) must have $E_L(L) \approx -0.15$ V. Accordingly, biomass properties of most kinds of tree leaves or needles in temperature climates (which are almost identical) dictate that ligand donor sites of litter be changed only slightly (if at all) during litter decay but the C backbone be cut down rather when metal recycling be efficient. Which

kinds of enzymes – containing which metals (if any are involved)? – can effect this?

2. with mykorrhiza absorbing the more plant-assimilated C the less P is present, there is an interesting situation as mobilization of many metals (alkaline earths, Zn, Al, Fe(III), Mn(III), REEs) should be the easier as phosphate levels are lower, thus less ligands would be required to retrieve the metals. Mykorrhiza, in turn, consists of heterotrophic organism (fungi) which absorb a "surplus" of citrato-, etc. metallates, degrading citrate and remobilizing metal ions the more the less phosphate is around. Thus in P-limited conditions substantial M^{n+} becomes available to the plants although by investing twice or more times than the 3% calculated above.

Furthermore, a minimum catalytic turnover can be estimated from the above ratio C/Mg ≈ 1,000, that is, how long chlorophyll and rubisco molecules must last before there is loss of Mg to the environment besides photodegradation (bleaching, occurs with chlorophyll) or reconstitution of the molecules, both by rainwater leaching of leaves still alive (Fränzle and Schimming 2008) and by litter-fall. Supplies and sinks are thus linked to the stoichiometric ratio once again which leads us to estimate it.

C/N in plant leaves ranges among 20–50 typically (Sterner and Elser 2002, cp. Table 2.16); in proteinogenic amino acids C/N is between 1.5 in arginine and 9 in phenylalanine and tyrosin, whereas it is infinite in cellulose and fats as either are N-free. For a complete plant, C/N uses to be <25. From this an "acceptable" (acceptable insofar as reproduction is not precluded) Mg autocatalyst inhibition by external perturbations or chemical destruction of agents by, e.g. competition can be deduced, such inhibition, e.g. due to amino acids, oxalate or malate competing for Mg with proteins that require it for function. This "acceptable" extent of perturbations in turn is estimated from the stoichiometric ratio of the involved elements, C (and sometimes N) being the backbone of the competing chemical species. Autocatalysis and thus metabolism will be maintained as long as ligand excesses – which bring about diurnal acid excesses in succulent plants due to malate (Heß 1999) – will not curb use of metal ions for enzyme activities by product inhibition. Obviously this situation depends on the relative complex formation constants $-\log k_{diss}$ of apoprotein and of some product ligand, combined with their stoichiometric ratio, with most metalloproteins containing but one or two (different) metal ions. It is impossible to make $-\log k_{diss}$ of the apoprotein arbitrarily large as it is given and limited by (the range of) electrochemical ligand parameter(s) of amino acid side chains and sometimes peptide bonds (often: 3 times imidazol + 1 heteroligand bound to Cu, Zn or Ni).

From Table 2.5 it can be seen that some quartet of possible donor sites inside a biopolymer or some multidentate ligand, e.g. an apoprotein, will only bind Mg in a tetradentate rather than bidentate fashion if (average) $E_L(L) < -0.05$ V, otherwise two of the donor sites will be "left out" (i.e., remain occupied by water) and thus be open for substrate acceptance; the corresponding value of $-\log k_{diss}$ is 3.6 in this equilibrium case, requiring $[Mg^{2+}] > 10^{-4}$ M/L in the serum. This compares to the binding constants of ligands derived from the tricarboxalate cycle as follows: $-\log k_{diss} = 2.8$ for monocitratomagnesate and 1.55, as noted before, for the malatocomplex (Furia 1972). Thus, if one assumes ≤ 50% of Mg binding sites maybe "wrongly" occupied by the products, then (C_6-)citrate/Mg be $<10^{(3.6-2.8)} = 10^{0.8} \approx 6$, while ($C_4$-)malate/Mg be $<10^{2.05} \approx 110$ and concentrations will remain limited in reality by both ligands being involved in highly active metabolic cycles so they will not pile up too much. Nevertheless just these two ligands imply that the stoichiometric ratio C/Mg be of order 1,000 at most, for $C_{citrate}/Mg < 36$ and $C_{malate}/Mg < 440$, notwithstanding the much larger C/Mg ratio of metalloproteins (in chlorophyll, C/Mg ≈ 40; N/Mg = 4 with $E_L(L)$ of the porphyrin ligand = 0 V (Lever 1990) while C/Mg ≈ 1,500; N/Mg about 250 for rubisco). Thus a total of some **4%** of carbon is directly linked to Mg in leaves of terrestrial plants as related to photosynthesis, while "free" aquated Mg^{2+} is rare and hydrolases, kinases and the Mg enzymes of the tricarboxylate cycle itself, etc. may have a share of some 20–30% (of course, this statement is not to suggest there are any Mg-C bonds like in Grignard reagents or cyanomagnesates but simply means organic compounds the functions of which (in photosynthesis) are linked to their containing Mg; actually we deal with Mg–N- (in chlorophyll) or Mg–O bonds (carboxylate, water, phosphorylated co-substrate, HCO_3^- in rubisco or PEP carboxylase) only). Thus some 70% of plant leaf Mg will indeed by linked to such simple organic acids, with tricarboxylate cycle intermediates being prominent which implies C/Mg ≥ 2.3 × (440 + 36), that is, exceeding a value of about

1,000. Such a condition, by the way, is also required to avoid precipitation of Mg oxalate or of struvite $Mg(NH_4)PO_4$ in the xylem.

The ratio between C and the metals essentially is that among C (in ligands like citrate) and the sum of the three most abundant (albeit weakly, yet complex-forming) metals Ca, Mg and Mn, making $C/\Sigma_{metals} \approx 200$. Alkali metals except of Li are reluctant to form complexes and thus can (must!) be neglected here.

The theoretical minimum of carbon "deviation" here would be 3%: the above ratio $C/\Sigma_{metals} \approx 200$ combines with citrate containing six C atoms to the statement that 3% of assimilated C must be "invested" given there were no retrieval of C after resorption of citratometallates. If, however, plants have to use >25% of their photosynthetic products just to mobilize metals from sediment (which implies most of citrate or malate is irreversibly lost to the soil as stoichiometry is constant) their chances to grow and reproduce at reasonable rates will shrink; anyway, they have no chance to organize a metabolism requiring larger metal amounts and smaller C/M ratios. Then, if there are alternatives like with hydrolases (Mg, Zn, Mn, Fe(II)) or in closing the N cycle by urease (Mn, Ni), the best will just be efficient enough to make a modest living. Green plants and chemolithoautotrophs change compositions of the C, N precursors in a manner as to increase metal affinities (complex stabilities) considerably, and the same holds for transformations of common S, Se and sometimes P compounds taken from the(-ir) environs. Conversely, the present stoichiometry of plants (and presumably also those of other organisms) does also reflect the "less-than-optima" listed in Table 1.1, plus the coordination properties of the metal ions actually used.

Formation of amino acids and their Mg complexes linked to the citrate cycle or/and oxalate reduction (the latter prevailing in plants) will not massively change the situation given C/N to be > 10. So we can indeed estimate an operative C/Mg ratio from complex (speciation) equilibria including the equilibrium conditions which imply activity of a metalloprotein, with the lion's share – some two-thirds – of Mg rather bound to low-molecular species. The observed ecological stoichiometry C/Mg ≈ 1,000 (which is universal except for marine algae which cannot avoid excess uptake of Mg) thus is an outgrowth of intraorganismic competition (among various Mg-binding ligands all of which contain C also). What would this equilibrium be like for other conceivable metal ion centers of a biological photocatalyst? Considering photoredox species which undergo, e.g. LMCT transitions as less suited, it is straightforward that any (other) redox-inert closed-electron-shell cation like Zn^{2+} (with Zn having about the same x_{2d} and x_{4d} values as Mg^{2+} but c_{2d} is considerably larger by some 1.2 units) should be exposed to the same kind of competition, at C/Zn ≈ 50,000. Here, a very small percentage only would stick to porphyrines or similar photoexcitable macroligands, too small to permit an efficient photosynthesis (nevertheless, metal ions like Fe^{2+} can replace water or sulfide as electron sources in photosynthesis of bacteria).

Nota bene, this statement that a metabolic cycle can tolerate about two-thirds of its autocatalyst be trapped elsewhere, just holds for Mg allocation in green plants and must not be taken as holding in general. On the other hand, it agrees with some low sensitivity (towards (in addition reversible) "blocking" of substantial parts of a biocatalyst element) of elements like Mg (or Fe, Zn) which can be expected from SNA given the large numbers of their functions: even then, cycles reliably remain strong, with citrato- or malatomagnesate forming some kind of "buffer" besides their being primary speciation forms just before and after root uptake. The total M/C ratio for complex-forming cations achieved by delivering citrate, malate, etc. to the solum is about 0.005, the mixture being dominated by Ca and Mg. If metal supply from the roots increases, plants use to respond to this by changing the patterns and ratios of oligocarboxylates (citrate, oxalate, malate) vs carboxamide (glutamine etc.) vs other amino acids (proline, alanine, glutamic acid) detected in root exudates (Marschner 1986), thereby also changing average complex formation constants as well as selectivities. In soil solutions and litter horizons, almost all proteinogenic amino acids (except of tyrosine and selenocysteine) can be identified but this does not at all imply that all of them are given away as such by roots or fungal mycelia. Rather, the considerable activities of proteases in topsoil (Bardgett 2005) cause this by local hydrolysis of entire proteins coming "from above" (litter deposition), with but few different AAs delivered directly beneath the soil–air interface.

Fluorescence observed with either chlorophyll samples or intact chloroplasts can be used to detect trace metals which also bind to porphyrines, competing against Mg^{2+}. REEs are well-known for their intense fluorescence with both aquaions, silicate matrices

(REE-YAG lasers) and also macrocyclic complexes, and their porphyrin complexes of kind $[M(porp)_2]^-$ are fairly stable (more so than that of Mg^{2+}). Accordingly, uptake of REE ions which make it up into photosynthetic organs should also be detectable by leaf fluorescence. Indeed, work on REE hyperaccumulator *Dicranopteris dichotoma* (a fern species) shows that among the REEs, at least Sm and Dy can and do replace some part of Mg in chlorophyll which brings about fluorescence typical of these f-block elements (Tyler 2004). Thereby, these REEs intercalate within the stacked chlorophyll (porphyrinoid; porp) molecules as to form $[M(porp)_2]$ sandwich complexes, the phthalocyanin (abbrev. pc) equivalents of which can be excited into electroluminescence even if the central REE ion is electrochemically entirely quiet, such as for $[Lu(pc)_2]$ which was already investigated for applications in corresponding devices. Likewise, REE- (Sm- or Dy-) exchanged chlorophylls will display ion-specific fluorescence if excited by NUV radiation, permitting an estimate of concentrations of REEs in leaves or needles. However, as a rule, BCF values of REEs for photosynthetic organs of vegetation are very low, of order 10^{-2} or smaller (calculated from Markert 1996; Kabata-Pendias and Pendias 1984), while that of Mg is of order 1, at a far larger outer (environmental, soil or freshwater) abundance. Thus, Mg cleavage from chlorophyll stacks within the thylacoid and energy quenching by ff or other excited states of REE^{3+} stay low in Ln^{3+} expositions of "higher" plants, limiting REE toxicities except in algae.

2.2.13.1 Plants Can Stand Some Soil Contamination

The readiness with which plants adapt to changing metal spectra in their environment (which is most important in their ability to set root on "extreme" sites such as serpentinite soils) in the above way is suggesting that, as a rule, metal "misallocation" can be tolerated within limited ranges only, proving "reserves" to be small in most cases, in full accord with the assumption that many trace (Ni) and ultratrace (Mo) metals do not execute much more than the minimum number of functions in plants (3) required by SNA reasoning.

Additionally, plankton algae display considerable variations in terms of stoichiometric ecology (concerning C/N- and N/P-ratios at least), unlike most other organisms which are highly constant in composition (Loladze et al. 2000; Sterner and Elser 2002). An analysis like the one done before on the C/Mg ratio can only be done if these principal ratios are not subject to appreciable variation in some given species. The (stoichiometric) C/N ratio in photosynthetic organs is about 30, while that C/Mg is close to 1,000; accordingly N/Mg ≈ 33, while N/Mg in chlorophyll itself is exactly 4 and the ratio/share in rubisco is about 300. Hence, chlorophyll accounts for some 12% of leaf or needle nitrogen only, the remainder represented by either simple ions or proteins while the share of dissolved amino acids or similar compounds usually is small.

With the interaction among tricarboxylate cycle and root activity, autocatalysis is traced to mobilization (sequestration, uptake) of metal ions by substances which in turn were synthesized by proteins containing and using these very metal ions. While the stoichiometric ratios remain rather large (for example, C/Mg about 1,000; much larger with heavy metals), the amount of organic compounds required to extract the metal ions from soil may almost outweigh the entire other metabolic activities in unfavorable conditions (some terrestrial plants use up to 30% of photosynthetic products for this purpose). This may be different with metallotrophic organisms such as iron bacteria. The ligands from tricarboxylate cycle more than outweigh all three metal ions (Mg, Fe, Mn); as noted before, the proper functioning – and thus the ability to retrieve these ions continuously – depend on the differences among complex formation constants. As stated in Eqs. 2.4 and 2.6, these differences depend on those of $E_L(L)$, c and x. Table 2.2 gives $E_L(L)$ values for many biorelevant ligands (citrate −0.25 V, malate −0.13 V and glycinate −0.05 V) which defines "meaningful" values of x. Considering polyphenols or hydroxamates in addition, the feedback between such siderophores and the metal(-loprotein)s needed to make them is similarly pronounced.

Generally speaking, the product must not completely abstract the metal ion from its enzyme position; thus any completely understood metabolic cycle – be it catabolic or anabolic – can be analyzed for the metal ions which might serve as biocatalysts here. We noticed before that $-\log k_{diss}$ (Mg) for the apoprotein must surpass that of oligocarboxylate complexes by >2 units which combines with $E_L(L) \approx -0.21$ V for oligocarboxylates to yield $x_{2d} \approx -2/(-0.21)$ or about +9.5. Mg with $x_{2d} = 8.24$ is close to this optimal value, as are other biorelevant

ions like Zn or Ni(II) which, however, are less abundant than Mg. Unlike with other ligands like phosphines or nitriles, there is considerable variation of $E_L(L)$ in mono- and dicarboxylates depending on substituents bound to (R3C–COO^-, e.g. −0.30 V for formate, −0.15 V for trifluoroacetate or −0.17 V for oxalate (Lever 1990) and −0.13 V for malate. This is corroborated by our work (Fränzle and Kollaske 2009, to be published) on redox electrochemistry of aqueous carboxylato- or phenolato- or amino acid-ruthenates. Neither Zn nor Ni is actually involved in the tricarboxylate cycle while Mn (unspecified oxidation state) and Fe occur in some other positions of the Krebs cycle only and the citrate/isocitrate relocation of OH group is effected by dehydration yielding aconitate and subsequent rehydration rather than employing Co (x_{2d} = 3.93 in aqueous Co(II), even less in species like cobaloximes or $[Co(CN)_5]^{2-}$) for an alternative C backbone rearrangement in this reaction step; presumably competition would become too pronounced once again. Biocatalysts can yet persist, providing the very high turnover numbers needed for efficient biocatalysis up to reproduction, in the tricarboxylate cycle at least only if competing complexation of metal centers by the product including their extraction from enzymes can be avoided or limited to some extent. Generally speaking, the volcano plot (Rothenberg 2008) of catalytic theory translates into a "fitness landscape" (Fig. 2.20) which extends orthogonally over the c/x plane.

In **marine (bio)chemistry** (Nozaki 1997; Fegley 1995) there is a commonplace classification of elements or specific speciation forms: Selectivity in marine element-biomass interactions is denominated by "nutrient" (n), "conservative" (c), "scavenged" (s) or redox-controlled (r) behaviors, respectively and corresponds to the ability of phytoplankton to *retain* some element after initial uptake which thereby opposes precipitation or aquatic (DOM, DON) complexation by (stronger) binding of biomass. Those "nutrient-type" elements, not all of which are "really" essential in marine biota, include Ni, Cd, Si, P, Se, the highest oxidation states of As and Cr, in addition Ag and light PGMs and all the REEs except for Ce but including Sc, Y, Ac (Nozaki 1997). What, then, means their being "conservative", "biotropic" ("nutrient type" – vertical or 3D-distributions be associated with that of plankton), "scavenged" or yet other, e.g. redox-controlled distribution in the oceans? Accordingly, biomass of marine phytoplankton does accumulate many non-essential elements like nutrients but does distinguish among oxoanions: the EO_4 oligoanions of S, P, Si, Cr and As – the last two being strong oxidants – display "nutrient type" behavior while Mo – also essential, also forming an EO_4 (di-)anion – does not. Although there is a slight trend from "scavenged" towards "nutrient" behavior in the 3d metal ions Sc through Zn, both among essential and non-essential metal ions, both metals with positive x_{2d} values (Be, Ni, Zn, Cd, Sm, Eu, Tb) and such with strongly negative x_{2d} (the other REEs, Sc, Y, La and Ac) *may* also behave like nutrients. Thus it apparently is no matter of affinity towards certain carriers which would transport molybdate (and are known to do so) alongside with the other oxoanions while cations (here, Cr is also taken to exist as some cation, i.e. Cr(III)) can interact with several different carriers also. In terms of SNA, this classification of elements exactly is about exit orders, then, making an average over many quite different kinds of marine phytoplankton: "nutrient" type behavior means low loss from biomass whereas losses increase as n < c < s, whereas r = redox-control cannot be given a general position in this scheme. However, Mg and Mo also show "c" behavior, presumably because either concentration (53 mmol/L for Mg and 100 nmol/L for Mo in seawater) is far in excess of plant demands (if washed with aqua dest until shortly before osmolysis commences, certain marine algae (e.g., *Palmaria palmata*) which also can sustain and grow in other, including hyperhaline media for some period of time (weeks), readily give away K^+ and appreciable amounts of Ca^{2+} but neither Na nor Mg).

The non-metal arsenic is an interesting case for its speciation (Santosa et al. 1994): As(V) is prominent at about 1 μg/L (10 nM) in ocean waters whereas As(III) (behaving conservative to scavenged) and methylarsenic species like dimethylarsinate or higher organoarsenicals like arsenobetaine, arsenosugars (Irgolic 1986; Thayer 1995) are much rarer (≤100 ng/L), and thus the dominant form is As(V) rather like P (inorganic phosphate). Arsenic probably is essential for many quite different marine organisms, including fishes and crustaceans (Irgolic 1986), believed to be linked to Zn uptake. As becomes enriched along P only in +V oxidation state; in marine creatures, there are often lots of lowly toxic organo-As compounds (Irgolic 1986; Thayer 1995; Emsley 2001). Likewise, there obviously is efficient retention of REEs in phytoplankton (cp. Weltje 2003 for limnetic counterparts) although the

ambient concentrations are 190 and 40 pmol/L (Y and La, respectively) and otherwise much smaller, ≤ 7 pmol/L except for Sc (15 pmol/L) and Nd (24). This means the retention by biomass is most efficient, BCF values of REEs being very high, like with limnetic plants (Cowgill 1973; Weltje 2003) and iron bacteria (Fränzle and Noack 2008, unpublished).

There are also Ba-dependent photoautotrophs: unicellular algae (desmides) which best grow in oligotrophic peat bogs poor in both humic substances and phosphate (Emsley 2001). There, N is scarce also but rather well bioavailable for both plants and aquatic animals (as a rule, there are very few fish species in such waters but lots of crustaceans). Some fishes can adapt to these conditions, like the tiny *Paedocypris* cyprinids (which inhabit bogs in Indonesia), the smallest vertebrates so far known at <8 mm total length which lack most of skeleton, apparently due to Ca supply being difficult there). Even though the functions of Ba in desmids are unknown, a SNA approach can be used to estimate and understand the rather peculiar effects which changes of hydrochemistry exert to population ecology and biodiversity of desmides when there arise chemical sinks (insoluble phosphates, sulfates). Both the cell number per liter of water and the biodiversity of desmides in bogs are almost reciprocally proportional to its content in inorganic phosphate, also decreasing with increasing pH or Ca levels (Woelkerling and Gough 1976; Brook 1987). Probably Ba concentration gets controlled by precipitation into apatite $Ca_{5-x}M_x^{II}(PO_4)_3(OH, Hal)$ with M = Sr, Ba, Fe(II), Zn, Pb, etc.

The response concerning cell number densities is simple if one assumes that the majority of dissolved Ba resides within the desmid cells (and must so for maintaining their metabolism) whereas that concerning biodiversity is less straightforward: presumably there are certain desmid species with a particular large Ba demand, followed by some continuum of lesser demands in related yet other species which combine to give the observed effects of Ba^{2+} levels on size and composition of desmid associations. Probably this corresponds to different numbers rather than kinds of biological functions of Ba in these peculiar algae. Though all the functions of Ba in desmides are unknown, its essentiality for them especially in difficult, low-nutrient sites like peat bogs is well established by deprivation culture experiments. It should be noted that there are also terrestrial plants enriching Ba considerably, particularly in nuts (Emsley 2001).

2.3 Some Remarks on Chemical Ecology

For biocatalytically active and thus essential elements understanding such losses described by behavior on an **ecosystems level** gives a first hint to the extent of "acceptable" losses. There apparently are elements distinguished by conservative or even scavenged (Mn) behavior and yet able to maintain essentiality, circumventing rather large losses, e.g. by high external concentrations (Mo, Mg). In real life, conditions are anything but constant: photochemistry will effectively change oxidation states (with Fe and Mn) and thus bioavailabilities of metals but with a diurnal interruption (possibly air oxidation of these metals and Ce in upper (<25 m) water column during nighttime) increases loss from an organism while AC orders may also respond to illumination in phototrophic organisms. Besides the alternation of day and night, there are other periodic (tidal changes in littoral or estuary regions) or non-periodic (casual aeration of anaerobic water-bodies) changes of conditions to which redox states of ambient elements will respond, producing (oxide precipitation) or removing sinks. What then will happen can be estimated from the Pourbaix diagram of the corresponding element (Figs. 2.21 and 2.22); but keep in mind the focus of Pourbaix diagrams is with thermodynamics, that is, things which might happen in absence or removal (catalyst, biocatalyst) of energy (activation) barriers, which need not imply or suggest that all this will really occur at any appreciable rate – otherwise neither the author nor the reader nor this book would be around in an oxidizing atmosphere, just CO_2 and water instead.

2.3.1 Constraints of Essentiality Caused by Consumers

Effects will be different, if there either is

1. No formation of precipitates or
2. The essential ion will be given away as such (aquaion) or coordinated to some ligand produced in the organism itself

Although in all these cases the exit order is going to increase, the chances of the organism to recover the element if conditions change again are different. Different sensitivities towards changing conditions, e.g.

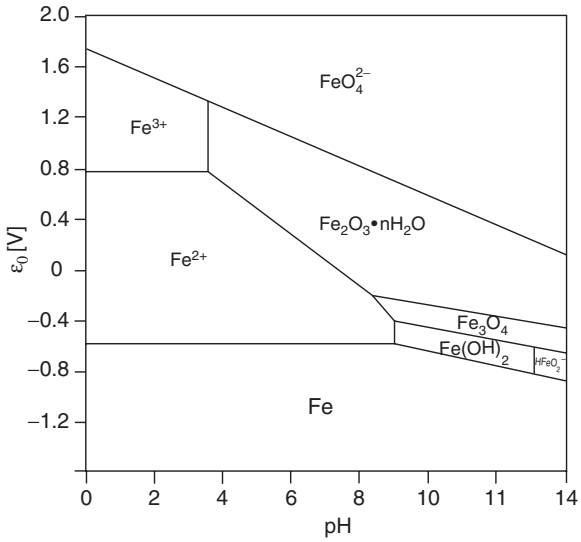


Fig. 2.21 Pourbaix diagram of iron (simplified, omitting protonated dissolved species such as $[Fe(OH)_{aq}]^{2+}$). The redox potentials of glucose and similar sugars (and most hydrocarbons also) at pH 7 lie flat within the stability regime of Fe^{2+}, hence Fe(III) can be used as a biological oxidant. Formation of insoluble oxides under O_2 atmosphere makes Fe access difficult for living beings, unless using reductants. Although magnetite Fe_3O_4 is stable in alkalinic media only, this reduction tendency of biomass components often gives rise to formation of magnetite nanoparticles in leaves and other photosynthetic organs (Fränzle et al. 2009). For the role of Fe in nitrogenases and the capability of non-stoichiometric Fe aquoxides and sulfides to reduce N_2 alike, consider the vicinity of the $Fe^{2+}/Fe_3O_4/Fe_2O_3$ triple point with the pH 7 N_2/NH_4^+ transition

P eutrophication, tend to change population compositions among present and possibly invading species when increasing exit orders make maintaining AC loops over generations difficult or else facilitate it. The response of desmid population compositions to Ca or P increases in peat bog waters by changing Ba saturation levels was mentioned above: whatever desmides do with barium, such having but 3–4 Ba-related functions would be viable only in rather acidic, "soft", almost P-free water bodies. Five or more functions would render corresponding desmides less sensitive; nevertheless such algae can hardly compete with other algae in rather eutrophic waters. The competitive exclusion principle postulates that unlike ways of using resources provide additional ecological niches and thus chances for co-existence. This can be accomplished either by different demands, using unlike metals to catalyze identical reactions or by different extraction procedures, that is, employing different electrochemical ligand parameters of the biomass. Nevertheless, an "exotic" agent such as Ba must get recovered from specific resources (food, soil solution or ambient waters) also, which is not trivial given that bond stability (Ba complexes are very weak as a rule) and ligand selectivity differ considerably (both c and x to the negative) from those elements forming the "window of essentiality".

Looking on aquatic creatures, including vertebrates (*Symphysodon* ("discus") and *Uaru* cichlids, salmonides like *Paracheirodon*) rather than terrestrial plants, there are fishes and plants which dwell in waters which are similar in many ways to peat bogs (very low pH, high concentrations of humic matter, only traces of metals), namely the "black-water" biotopes of Amazonia (e.g. Ladiges 1984). These plants and animals apparently are not distinguished by demands for exotic bioelements as desmides do; at least it is unlikely that they (like desmides) make use of elements which are most susceptible towards precipitation and mineralization of ions or molecules because aquarium care experience has it that most of these fishes and plants do also reproduce in much harder waters.

Now turn to a complete plant as it chemically interacts with the surrounding ecosystem: in roots metal ions, anions and water are abstracted from soil solution and several mineral phases (Figs. 2.6 and 2.13); the ligands from the tricarboxylate cycle are both small and not too selective. This partial system is autocatalytic with respect to Mg and Fe since enzymes containing these metals are in turn involved in Krebs cycle and thus afford the species then tapping external Mg and Fe reservoirs. The further way was already discussed; suffice it to say here that water transport driven by evaporation in the canopy brings about concentration, the more so as the volume (living biomass) of canopy photosynthetic organs is considerably smaller than the complete volume (and water mass) of the rhizosphere. Hence, as the equilibrium is shifted towards metal complexes upward in the plant, increase of metal ion concentrations becomes a spontaneous chemical process if $-\log k_{diss} > -1$, and this even holds for metals like Ba. When cytosol metalloproteins are more stable than citrato- or malatocomplexes of the very M^{n+} ions, transport is enhanced additionally. As $E_L(L)_{malate}$ is (calculated to be from complex stabilities) −0.13 V, less so for citrate, and all the biorelevant metal ions except of Mn(II), Ca and Sr have positive x_{2d} values, it is straightforward to assume that $E_L(L)$ for the carrier proteins be larger than this value, implying a role for ligand sites other than carboxylate (aspartate, glutamate residues), however, bear

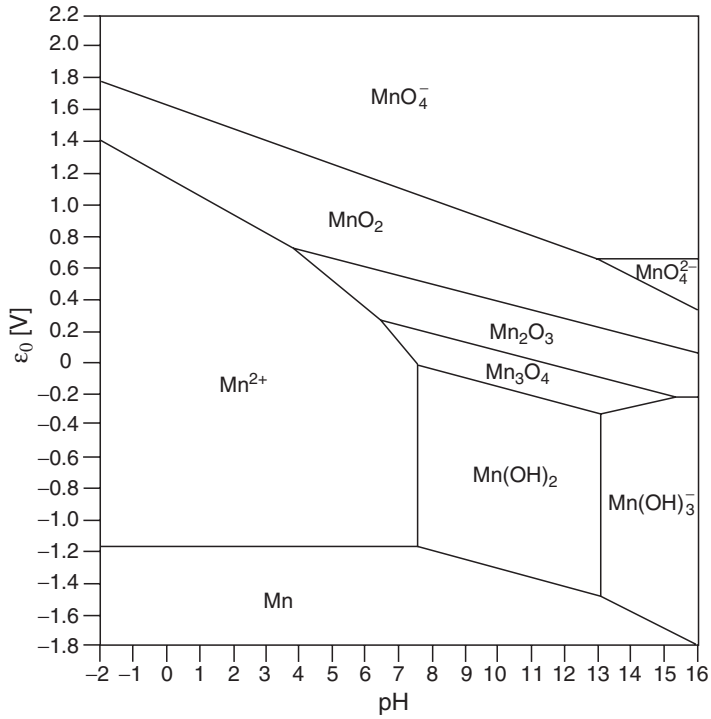


Fig. 2.22 Pourbaix diagram of manganese. Like with Fe, it is apparent that Mn^{2+} will be stable coexisting with glucose and similar biological reductants. Formation of insoluble oxides under O_2 atmosphere makes Mn access difficult for living beings, unless using reductants. The MnO_2/Mn^{2+} connection line corresponds to a powerful means of catalytic oxidation when exposed to O_2, permitting both oxidations of inorganic species like Cr(III) (mobilizing and "toxifying" chromium), Ce(III) or chloride (Deacon process of Cl_2 production) and degradation of – even polymeric, like lignin – phenolic and other biogenic substrates by Mn-exoenzymes fungi deliver into soils

in mind the changes are accomplished by just removing the primary (sequestration) ligand in the manner of oxidative exchange. Hence, although x_{2d} is positive for most of the metals, $E_L(L)$ of secondary carriers (notwithstanding equilibria between bi- and polydentate behavior towards some of the metal ions which might occur in a (carrier) protein, often around −0.05 V (for Be, Mg, Al, Fe(III), etc., cp. Tables 2.5 and 2.6)) need not be larger than −0.13 V. It is interesting that almost all hyperaccumulators, most of which enrich Ni (Farago 1986, Vernay et al. 2006), contain very high levels of histidine in xylem liquids. Free histidine bears an imidazol moiety ($E_L(L) = +0.12$ V) but, like other functionalized amino acids, its complex formation constants with various metal ions are very similar to that of simple glycine or alanine, suggesting an identical way of binding. Possibly in hyperaccumulators there are peptides, which like albumin (two imidazols, two carboxamide functions at the same one terminus) contain much histidin in a position exposed to metals.

Generally speaking, in both plants and other organisms autocatalysis occurs on quite different levels of hierarchy. In ecosystems such as forests there are also interactions among different plants, (mainly organized by) mykorrhiza, and other fungi, transporting metals (and nitrate, various organics) from one to another or competing for mineral resources. If there is precipitation of metals such as Mn, the topology of the network may change also, inverting its way of response to yet other, secondary perturbations. It should be noted that all data taken on plants, etc. from the native environment (Markert 1996; Fränzle and Schimming 2008, etc.) are by themselves influenced by such interactions, although the differences observed among co-existing plant species demonstrate that "smearing" is rather limited. Of course, even if this were more pronounced, the more fundamental criteria such as the three-functions-rule are not touched or altered but still in operation to be "obeyed". Empirically, the confinement of the essential elements to but a small region in the c/x map (Fig. 3.1)

is not levitated, the less so as apparently grassers are capable of limiting the numbers (and thus indirectly the ranges of chemical properties) of essential elements in producers, including green plants. The only parameter which changes indeed will be efficiency of supply/input (resorption) and geobiochemistry of certain sinks, that is, exit rate rather than exit order.

On the top levels of ecosystems and biocoenoses, however, there is no autocatalysis anymore as these systems do not (cannot by definition) reproduce. Rather, they form sources or sinks for elements, acting as a kind of "environment" for elements autocatalytic to at least some members of the embedded biota. Including the case that some essential elements become deposited (Fe by iron bacteria, Ca by corals, etc., S by sulfate reducers) or transferred to the atmosphere, thus vented in an either more (Se-, As polymethyls) or less reactive (N_2) form, thus increasing exit orders. With always but a part of the metabolized element mixture being locked up in biomass over longer periods of time, the situation is "asymmetric" on each trophic level. For first-level autotrophs (producers), sufficient admission of substrates may become an issue even though assimilation rates of 40–100 mol C $m^{-2}a^{-1}$ may be reached (Bardgett 2005), as an increase of CO_2 in the gas phase brings about increased growth rates of plants. Moreover, there may be limitation by almost any of the essential elements, thus the importance of using fertilizers. Sinks are less important than sources here while on higher (heterotrophic) levels the situation will be reverse, often causing concentrations of metals to decrease from one trophic level to the next (Fränzle and Markert 2002b; Sterner and Elser 2002). Thus for plants the focus may be placed on uptake challenges and thus on coordination chemistry for the dominant metabolic cycles – that is why Mg was selected for discussing an example (Fig. 2.18).

This ecological argument – that animals and other heterotrophs control and in effect cut down the number of elements which may be essential for those autotrophs they feed on, excluding the likes of V, Cr or Co – should have hold from the very moment when heterotrophic metazoans evolved, some 1.3 bio. years ago. But even for the uppermost Precambrian, there cannot be argued about trophic relationships as there were no hard protective structures, indicating there were no larger 2nd- or higher-level consumers yet. Moreover, it remains entirely open to speculation even whether Vendobiota were auto- or heterotrophic (Seilacher 2008), so we can extend this argument backward to the Cambrian at best, that is, much after separation of (last common ancestors of) multicellular (photo-)autotrophs and heterotrophs had taken place, with the paradoxical result accounted for by stoichiometric network analysis. The situation with Ni – sometimes presumed to be bound to "vanish" from the list of essential elements also as a long-term consequence of changing global biogeochemical conditions (Williams and Frausto Da Silva 1996) – is different; it has a better standing than might be presumed since it is involved in the N cycle with plants (whereas animal and bacterial ureases rather operate on Mn) and in CO reduction to CH_4 and acetate with archaea and clostridia, promoting several reaction steps in either case.

2.3.2 Trophic Nets

2.3.2.1 Concentration Effects, Variation of Transporter Ligands and Fractionation of Elements in Trophic Chains

While also sequestration and absorption of metal ions by terrestrial plants or fungi do mainly depend on complexation equilibria next to the root system or mycel, respectively, the local pattern of ligands in the soil and of anions forming hardly soluble salts usually is not known precisely enough as to estimate the actual state of equilibrium (distribution); the situation is better for aquatic organisms (both p1ants and animals). The latter plants and animals get very close to distribution equilibrium (Paquin et al. 2003), though there is active transport of electrolytes in either direction. In addition, the "classical" ratios of ecological stoichiometry, that is, C/N and N/P of aquatic organisms were most intensely investigated (Sterner and Elser 2002). C content is fairly constant (42–52%) except for some bacteria, whereas that of N contents vary between about 0.8% and 6%. Accordingly, variation in C/N is about as large as that concerning N contents itself. The situation is similar with P and thus also for the C/P ratio. Stoichiometric (not mass) ratios C/N, C/P and N/P in turn depend upon the stoichiometric ratios of ligand-forming species which in turn differ with respect to $E_L(L)$.

Concerning the donor sites, N-containing ligands such as amino acids, peptides, hydroxamates or N-heterocycles bring about much higher electrochemical

ligand parameters (−0.05 up to some 0.25 V) – and thus pronounced affinities towards metals like Cu, Zn, Al or V – than C- (carboxylate, not CO or isocyanide) or P-based (phosphate esters, nucleic acids) (about −0.2 downward to −0.3 V). Given the fairly extended biological range of C/N and N/P (implying an additional effect by C/P) will control the bioaccumulation behaviour of metal ions which may bind to either kind of ligand, with the effects possibly augmented throughout some trophic chain, concentration and bioaccumulation effects can be attributed to some variation of effective electrochemical ligand parameters along this trophic chain (cp. Eq. 3.3). They can likewise be quantified (but take regard of the rarity of ongoing accumulation of elements in a "minimal" (phytoplankton/zooplankton/planktivorous fish) limnetic trophic chain, which is mainly restricted to niobium (Fränzle and Markert 2002b) while usually attenuation and accumulation occur one after the other with the same metal ion). The exciting perspective which arises from this is the chance to define some direct link between data from ecological stoichiometry (namely, C/N and N/P ratios) and (intraorganismic spatial) distributions as well as functions of metal ions.

As far as biochemistry is concerned, substrates or reaction partners of catalytic cycles are not arbitrary but must belong to the set of biomolecules with their corresponding functional groups which in turn determine values of $E_L(L)$. In biology there are many complicated if not even polymeric ligands in which the electrochemical ligand parameters of different functional groups are subject to considerable variation. Empirically, one cannot tell these apart by binding them to ruthenium(III), expecting each would give rise to some individual peak of Ru(II/III) or Ru(III/IV) potentials but rather, like with humic acids (Fränzle and Kollaske 2008, as yet unpublished) one broad signal is produced even though there are examples of linkage isomerism (Burmeister 1968) with many different biomolecules, including "classical" (pre-1970) cases such as cysteine, methionine and several nucleoside bases (ibid.). Upon changing either oxidation state of one given metal or the very metal ion itself, the sign of x may change for bidentate complexation, causing, e.g. inversion of preferred binding sites in linkage isomers (Balahura and Lewis 1976; Bütje 1987). In biochemistry, this enables selective activation of some substrate/product couple for either direction of the reaction (say, hydrolases/ligases) or attacking some specific functional group within such polymer with a minimum of product inhibition for either case. However, both ligand sensitivity x and its sign are specific for the given biocatalytic ion (i.e., are constants typical for that material), there will be specific attack upon certain functional groups of a polyfunctionalized macromolecule in the highly selective manner so typical of enzymes rather than speeding up just every kind of reaction which might occur. As a result, certain metal ions would not be suited as enzyme central ions. In effect, this is how the values of parameters c and x are linked to the Biological System of Elements, giving a theoretical backbone to plots like the mappings in Figs. 3.1 and 3.2.

Intrinsic binding stability c and ligand sensitivity x describe the selective interaction between some metalloprotein and its substrate, bringing about selectivity. Other functional groups or ligand molecules either would not undergo coordination at all or block the enzyme altogether (e.g. cyanide, redox-inert Zn(II)). Thus, using metal ions in proteins having "appropriate" c and x alone might not accomplish high levels of selectivity in catalytic transformations but the environment – in addition providing stereochemical information – must discriminate against certain toxicants which block the enzyme altogether. However selectivities of both cation sequestrants and anion transporters are poor at best. Sequestrants use to display some affinity towards multiple metal ions, acting as ligands and paving way for entrance of non-essential elements. This gives rise to accumulation of chemical elements also beyond the necessities of biocatalysis, "beyond" referring to both the amount and number of different metal ions; yet, the effects differ depending on the concerned metal ion(s), species and organ. Even though some metalloproteins may operate on different metal center ions (e.g., Zn vs Co), substrate selectivities and relative turnover rates tend to change (Vallee and Williams 1968) then.

Quite in the same manner as selective metal ion affinities are attributed to the electrochemical ligand parameters of their binding partners, and electrochemical ligand parameters of biomasses may be calculated from c, x and the corresponding complex formation constants, it is feasible to define some effective electrochemical ligand parameters of the respective biomass, treating the biological sample as if it were chemically homogeneous, consisting of but one single ligand. The corresponding calculation is based on known data of relative enrichment, that is, on identical (or, practically speaking, next to identical) BCF values for some cluster

of chemical elements (preferably, not or not only essential ones). Given a known BCF value set, the basic equation is rearranged as to provide an effective electrochemical ligand parameter making use of data for different ions with identical BCF values for the same organ, say, birch leaves. The c and x values for the involved ions are linked by linear regression once again. Assuming that identical BCF values correspond to identical complex stabilities, this electrochemical ligand parameter is used as a proxy or shorthand for the specific metal–biomass interaction behavior, acknowledging this interaction to be shaped by coordination chemistry mainly (Figs. 2.21, 2.22, 3.1 and 3.2).

Thus after the Cambrian "explosion" in novel trophic chains with now >2 trophic levels the issues of nutrient transfer and cycling, including that of trace metals, became prominent: even now, there are no essential metals which undergo consistent, step-by-step enrichment within limnetic trophic chains (Fränzle and Markert 2002), the only metal to do so from phytoplankton via zooplankton eventually to piscivorous fishes being niobium. In addition, the shift from uni- to multicellular top-level consumers implied cell-type differentiation and thus phase rules to become operative, once again changing the "allowed" patterns or multiplicities of essential elements or cofactors.

Yet, even in some recent biocoenoses the rule of top-level predators may still be taken by protists, e.g. in the life communities of sewage treatment plants, but apart from being somewhat artificial (the environmental conditions created there do not effectively exclude survival of insects or even fishes – certain species of loricariid catfishes are routinely bred and raised in pools constantly "nutritioned" by raw sewage sludge in both Florida and Eastern Asia) this does prove that in protists – and probably other unicellular organisms – there can be sufficient control of both element intake paths – via food and water – without resorting to either specialized biochemical structures such as stomachs or gills or relying on phase rules implied by unlike cells. This does mean that the SNA description of autocatalytic and thus also reproductive processes reveals sufficient criteria to "organize" element budgets and use patterns along longer trophic chains with no metazoans necessarily involved in consumption/predation.

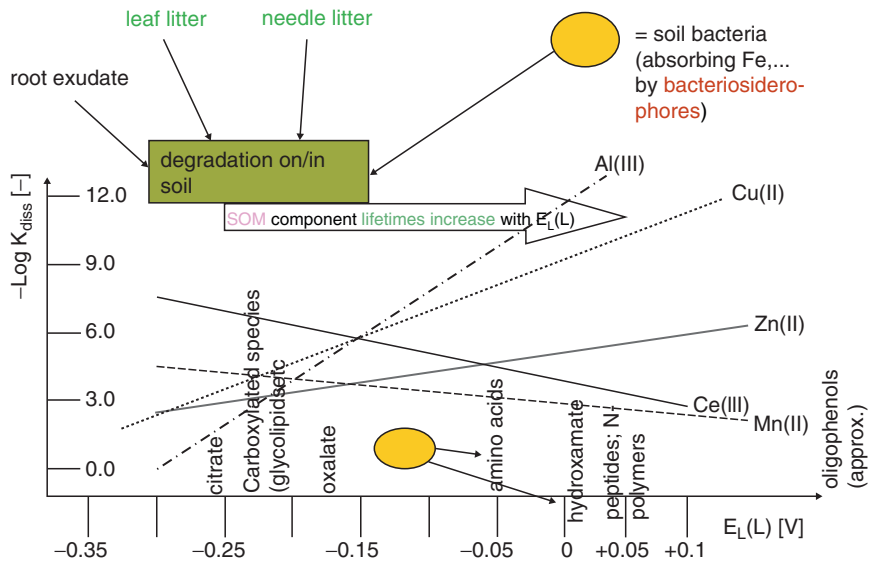


Fig. 2.23 During early degradation $E_L(L)_{eff}$ of the leaf litter is kept close to –0.15 V allowing for unfractionated "recycling" of metal ions which got thereby back to the soil interface. Rather than modifying functional groups, C/O (carbohydrate) or C/N (protein) or C/C (polyphenol) backbones (*extended lines*) are cleaved by hydrolysis or oxidation (*products with broken lines*) while the functional groups (*squares linked to the line(-parts) perpendicularly*) remain active towards metal ions (*round dots*) rather than being altered into other functional groups (*triangles linked to the line(-s) within short time*) (which happens much later). In temperate deciduous forests, degradation even starts before littering: during leaf senescence in autumn, some 30% of the Ca content of a leaf may already be leached before it even falls to the ground. Hence, weak complexes ($-0.15\ x_{2d(Ca)} + 0.73 \approx 2.4$) will break down soon after first, apparently marginal chemical changes (slight increases in $E_L(L)$, cp. Fig. 2.9).

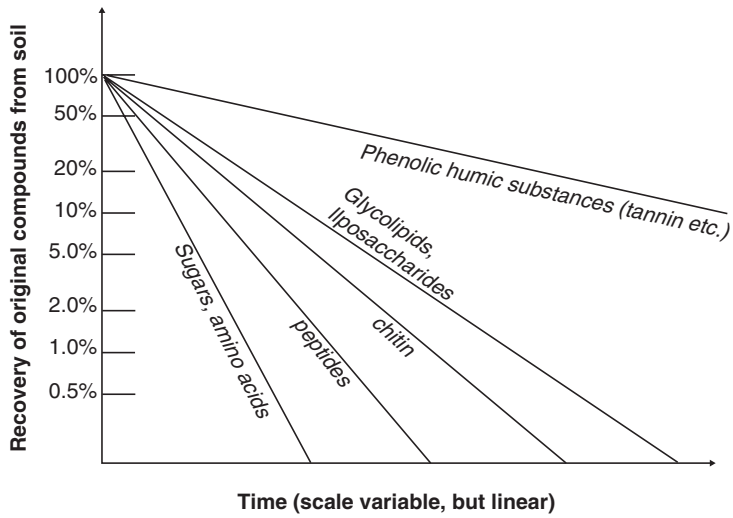


Fig. 2.24 Degradation kinetics of various soil organic compounds. Here, just removal of the original compounds is considered which need not include or imply cleavage of the functional groups binding metals; e.g. during saponification of the phospholipids which are highly abundant in fresh litter and young soil materials a- or monolipidated glycerol phosphates are produced which have identical complexation properties. Hydrolases in litter and soil also attack proteins, producing peptide fragments or single amino acids which means that binding by AA side chains will be replaced by the usual N,O-chelation of aminocarboxylates. Whether there is substantial change in $E_L(L)$, being −0.05 V in the end, depends on the AA sequence of the (outer parts of the) former protein: strongly basic (lysine, arginine) or acidic (aspartic and glutamic acids) proteins will display large ($\Delta E_L(L)$ about ±0.15 V) changes as do cysteine-rich ones while usual sequence will not give rise to any larger changes, thus permitting for easy recycling of metal ions. Time scale is linear but will depend on climate, humidity, ... As a rule, lifetimes of sugars (some 37% left over) in temperate-climate soils are of order weeks to months (modified after van der Valk 2003, using additional data from Gleixner et al. 2001)

Once we acknowledge this, we should proceed to ask whether an "upgrade" to three- or more-level trophic chains in the Cambrian also might have had an impact on the number of essential elements (usually larger in animals than in plants or bacteria) which is/was due to selection criteria from chemistry rather than those derived from "pure" SNA. These selection criteria might include the chance to obtain some element by grazing a biomat or catching prey up to an extent which allows for its eventual biochemical or biocatalytic use.

2.3.3 Succession and Ecological Stoichiometry Including Intermetal Ratios

Living beings are through-flow reactors the metabolism of which implies some constant exchange of matter with their environments. Both living beings and the catalysts of which they are composed will multiply during livespan; this holds downward to single kinds of molecules and all hierarchical levels of organization in between. Accordingly, functional forms of essential elements, such as metalloproteins, are reproduced, too before (especially in metazoans) or/and during reproduction. Although this holds up to organisms, higher hierarchical levels of biology do not behave in the same manner:

Ecosystems do not reproduce (nor will there be any "cross-breeding" along ecotones), yet, their internal and outward-directed matter flows may be analyzed by SNA, too, as they include "covered" autocatalysis (in the individual organisms), with the matter-flows controlling the behavior of the systems. Here, differences between simple, linearly catalyzed or non-catalyzed transformations on one side and autocatalytic ones which is stressed by SNA will "carve out" biological processes within biogeochemical networks (Fränzle 2000). (Reasonable amounts of) some essential element will promote growth of such organisms which need it, with the result that an increasing share of this element will be tied up in biomass, or, as Vernadsky did put it around 1930:

"living beings or the biota as such will tend to integrate and use all of the bioavailable atoms of some element within biomass (first biogeochemical rule)" (Levit 2001); in our terms: The extent of biogenic

autocatalysis will increase with time going on (Vernadsky's argument then, however, was based on quite another line of reasoning). Vernadsky's postulate now can be compared with modern data on the share of essential and some non-essential elements bound to the living biota as compared to other speciation forms in those layers of Earth accessible to organisms (air, ocean/freshwater, topsoil).

For carbon, about 1.7% of total C in this column, including CO_2, CH_4 in the atmosphere and carbonates in ocean, soil, are fixed in living biomass (some 610 Pg (gigatons) which is a global average of 1.2 kg/m^2), for N, Mg, Mo and S this is of order 10^{-3}%. For certain essential trace metals also up to several % are bound to biomass (e.g. Cu and Fe), thus Vernadsky's statement on "pumping" of the utmost possible amounts of (all?) the trace metals into biomass called the first biogeochemical rule (Levit 2001) must be modified, acknowledging the limits set to composition of biomass by stoichiometries of coupling among different enzymes. Only **three elements are integrated** into biomass **by some 50% of** the **total** amounts in atmosphere, hydrosphere (including oceans) and topsoil, two of which are essential (Zn, P) and the other rare yet highly toxic (Be) (Fränzle 2008; habilitation lecture given at Vechta; 14 February 2008, see Table 2.22 below), whereas essential elements C, Cu and Mn (+ Fe) partition into biomass by ≈ 1–10% (the value from higher organisms given for Fe content in Table 2.22 is smaller than 1% actually but consider the tremendous enrichment of Fe and considerable abundance of Fe-processing (both –reducing and oxidizing) bacteria). Actual use of elements in biomass thus as a rule remains rather limited (or living beings are

(a) Unable to retrieve most of N, S, metals from minerals for solubility (or other thermodynamic) reasons or
(b) Given constraints for "meaningful" organismal compositions, the limited amounts of C accessible to organisms in turn set limits to organismic intake of all the other elements, notwithstanding the large remaining atmospheric pools of both C and N)

In soil evolution, N enrichment first (in early stages) enhances initial decomposition of litter but suppresses humus decay in later stages. Accordingly, the rate of shift of $E_L(L)$ changes during soil aging also depends on the C/N ratio which, as a minimum, initially must be that in the leaf/needle litter itself, that is, something like 20–50; in the end, it tends to settle at 29. It is not apparent from the end what would happen if, by some combination of litter composition and N input, C/N would be 29 already but, given that C is returned to air (as CO_2) rather than N, the latter stages of development would take benefit of a prior C/N > 29. Initially, litter degraders might be N-limited (C/N in bacteria often is <10) unless there is additional input, or nitrate directly goes into nitrogeneous oligomers directly after reduction or along it, possibly by direct (enzymatic or

Table 2.22 Amounts of elements (per m^2) in biota, atmosphere and the oceans, representing the entire column of the biosphere, from upper troposphere through surface-covering waters (70% of entire Earth surface!) downward to life-inhabited upper soil and sediment layers. Three essential (P, Zn, Cu) and one highly toxic (Be) element are gathered in biota by 8.5–43% of the entire inventory while others except of C, Mn (1–2%) remain most outside of biomass.

Element	Biota (mol/m^2)	Atmosphere (Mol/m^2)	Oceans (mol/m^2)	Ratio biota/biota + biospheric environment [-]
C	100	130	5,600	0.017
N	6.4	545,000	1,575	0.000012
S	3.4	0.01	70,000	0.000048
P	2.3	0	5	0.32
Zn	0.01	0	0.014	0.42
Cu	0.00057	0	0.006	0.085
Mn	0.013	0	0.9	0.014
Fe	0.010	0	1.35	0.0072
Al	0.011	0	2.8	0.004
Hg	0.000005	10^{-8}	0.0018	0.003
Be	0.0000004	0	0.00000053	0.43

Table 2.23 Complex stabilities and electrochemical ligand parameters for humic and fulvic acids, assuming bidentate complexation

M^{2+}	$-\log k_{diss}$ (humate)	$E_L(L)_{humate}$ (calculated)	$-\log k_{diss}$ (fulvate)	$E_L(L)_{fulvate}$ (calculated)	Difference
Cu	8.65	−0.02	4.0	−0.23	
Cd	6.25	+0.32	–	–	
Zn	5.72	+0.07	3.6	−0.18	
Average	–	+0.12	–	−0.21	0.33

spontaneous) nitration of polyphenol/lignin aromatic rings much in the way nitroarenes such as nitronaphthols form in rainwater. Then anilines the rings of which in turn are readily cleaved by haem peroxidases ($Fe^{IV}O^{2+}$ porphyrines) form by reduction and may convert into amino acids directly, N input rendering polyphenols nutrients and C sources likewise.

Given that C is returned to air (as CO_2) rather than N (which ends up as NO_3^- again rather than N_2, N_2O, etc.), the latter stages of development would presumably take benefit of a prior C/N > 29. In anoxic soils, the very large (>>29) C/N ratios inside anaerobic organisms which make use of poorer oxidants than nitrate, Fe(III), e.g. of sulfate. Initially, litter degraders might be N-limited (C/N in bacteria often is <10) unless there is additional input, or nitrate directly goes into nitrogeneous oligomers directly after reduction or along it, possibly by direct (enzymatic or spontaneous) nitration of polyphenol/lignin aromatic rings much in the way nitroarenes such as nitrophenols and -naphthols form from NO_2/nitric acid in rainwater (Belloli et al. 2006). Then anilines form by reduction, the rings of which in turn are readily cleaved by haem peroxidases ($Fe^{IV}O^{2+}$ porphyrines) and may convert into amino acids directly, thus N input as NO_x renders polyphenol nutrients and C sources likewise.

Concerning the soil-borne ligands, $E_L(L)$ of both humic and fulvic acids can be estimated from "conditional" (aqueous, pH ≈ 5.5, low ionic strength) complex formation constants (data from Scheffer et al. 1998).

Once again, an increase of $E_L(L)$ upon aging of soil-borne organic matter can be observed. Corresponding binding sites which were (at least suggested from IR spectroscopy, ^{13}C-NMR to be) abundant in humic/fulvic acids include phenolates, carboxylates and β-ketoenolates, with formation of the latter upon partial (photoassisted?) oxidation of fulvate phenol moieties being quite conceivable. With humic acid, there should be pronounced retention of elements like Cu, Ni by soil whereas $E_L(L)$ of fulvic acid is so low that the Irving–Williams series will undergo some changes. On the other hand, $E_L(L)$ of fulvic acid is rather close to the values for leaves/needles of many terrestrial plants of moderate climates. This may be due to the origins: fulvates are produced by leaching of litter mainly without substantial chemical changes in the corresponding organic fractions except for loss of very labile ones such as sugars. Our own work on Ru complex electrochemistry in aqueous medium (Kollaske 2009; Fränzle and Kollaske 2009) dealt with both low-molecular (M ≈ 2,500) humic acids and models of phenolic carboxylic acids (salicylic, caffeic, gallic acids), providing the following aqueous potentials which correspond to a CH_3CN-solution potential of about −0.05 and +1.2 V vs SCE, while the series of first observed potentials invert the Lever scale (Table 2.24).

Whether there is substantial change in $E_L(L)$, being −0.05 V in the end, depends on the AA sequence of the (outer parts of the) former protein: strongly basic (lysine, arginine) or acidic (aspartic and glutamic acids) proteins will display large ($\Delta E_L(L)$ about ±0.15 V) changes as do cysteine-rich ones while usual sequence will not give rise to any larger changes, thus permitting for easy recycling of the metal ions from litter or young soil layers. The precondition for this to happen even before complete soil peptide hydrolysis had occurred is an increase of binding stability, depending on the mixture of amino acid side chains and metals alike (after degradation to amino acids which are, moreover, short-lived in soil, the side chains do no longer appreciably influence complexation (Irving and Williams 1953; Kiss et al. 1991, Sövago et al. 1993)). Now consider a mixture of two or several different ligands interacting with various metal ions of unlike x values, possibly – but not necessarily – including toxic ones. These ligands are present in soil as depending on its being supplied with novel (plant) organic material (litter) or soil degradation: The organic compounds in soils which were derived from plant (leaf, needle, wood) litter considerably differ with respect to their lifetimes and persistence:

(mono- and oligosaccharide) sugars ≈ free amino acids < proteins < chitin < (glyco)lipids < lipopolysaccharides < tannines, lignine (Gleixner et al. 2001)

Table 2.24 Aqueous potential values of biogenic ligands (Ru complex, pH 1.7) and Lever scale

Ligand	E_{aq1} (Ru) (mV)	E_{aq2} (Ru) (mV)	$E_L(L)$ (mV)
Acetohydroxamate	−269	+903	−200
Catecholate	−260	901	−280 (Rocha et al.)
Malate	−237	899	[−130] (calc.)
Glycinate	−291	908	−50
Oxalate	−231	900	−170
Salicylate	−265	900	−70
Caffeate	−269	901	–
Gallate	−241	901	–
Humate	−154		–

All of these species, except for fully esterificated lipids, display ligand properties towards metal ions and hence influence speciation of the various metal ions in soils. The behaviour, as usual, can be described by their corresponding electrochemical ligand parameters $E_L(L)$, with tannines and lignine prominently bearing phenoles, with lignine polyphenols being derived from hydroxylated or methoxicinnamyl alcohols. The respective $E_L(L)$ values are:

(glyco)lipids (ligand activity (if any) depends on presence of free carboxylate groups [$E_L(L) \approx -0.25$ V] or esterification extent) = lipopolysaccharides (same as glycolipids) < (mono- and oligosaccharide) sugars (−0.11 V) < free amino acids (−0.05 V) < proteins (+0.03 V (carboxamide moiety, possibly modified by side chain-ligands from amino acids)) ≈ chitin < (+0.03 V (N-acetylamino group)) < tannines, lignine (phenolate ≈ +0.23 V) (Figs. 2.9 and 2.16; Table 2.2)

Except for glycolipids and lipopolysaccharides which are moderately persistent in soils but their complexation behaviour depends on hydrolytically "liberated" carboxylate moieties, the sequence of increasing $E_L(L)$ in biogenic soil organics coincides with that of increasing persistence. If there still is a "mismatch" between the results of redistribution and ligand transformations and the requirements of the plant society now living there, the present vegetation is going to be at least partly replaced, with obvious consequences along the food-chain, by other plants which differ in metal-uptake, both concerning essential and possibly toxic ones. This then will be attributed to either pollution or succession by observers (botanists, ecologists), either perceived process inevitably bearing consequences for animals feeding on these plants. In extreme cases (very high burdens of metals such as Zn), only a highly tolerant flora, containing very few species, may persist at the site (cp. Fig. 2.19a–c). This argument on refractiveness and ligand properties of (heteroatom-containing) organics getting into soil and the effects of biogeochemistry on these over time directly takes us to the issue of succession, e.g. in aging and drying peat bogs.

2.3.3.1 Stoichiometric Changes During Succession

As an example for effects of **succession**, consider the sequence of plants growing in one bog which continuously converts into solid land: first, there will be aquatic plants, with *Sphagnum* species growing on the surface. Then some birch trees (*Betula* spp.) will grow over this, with *Sphagnum* getting replaced by moisture-loving grasses such as *Molinia caerulea* and then heath-scrub species like *Vaccinium myrtillus* once the former bog got dry on the upper edge. Eventually pine trees take over in the now dried region. Typically, odd (reactive) nitrogen is scarce there, giving rise to carnivorous plants such as sundew in old bogs, followed by establishment of species which can absorb free amino acids like glycine as such from soil (or overlying mosses) besides nitrate or ammonia (among them, the above *Vaccinium myrtillus* (Bardgett 2005)). The high levels of humic acids control plant (-root) access to elements like Cu and Fe. The principal stoichiometric ratios are as follows, including Mg/Mn and those of elements involved in redox metabolism (Mo, Zn, Cu, Fe) and nitrogen cycling (Ni), using the BSE data obtained for plants from Grasmoor bog (Fig. 2.25) near Osnabrück (Lower Saxony, FRG; Markert 1996) once again (Table 2.24).

The decrease in Fe/Cu ratios points to changes in either element retrieval or the altering difficulty to modify (a) phenolic soil components and (b) hydroxylate aromatic organics. Once there is "dry land" (starting with birch, from the left), Mg/Mn almost remains constant (Fig. 2.26).

The ratios of other oxidoreductase centers to Cu abundances represent the relative importance of oxidations of phenols and related compounds while Mo is involved with redox transformation of non-metal oxospecies mainly. During succession, Mo levels and Mo/M′

Fig. 2.25 Partial view of Grasmoor bog near Bersenbrück, Lower Saxony, Germany (North-West). The 33 ha bog area is distinguished by two ponds surrounded by the various stages of succession in a bog slowly falling dry (Markert 1996)

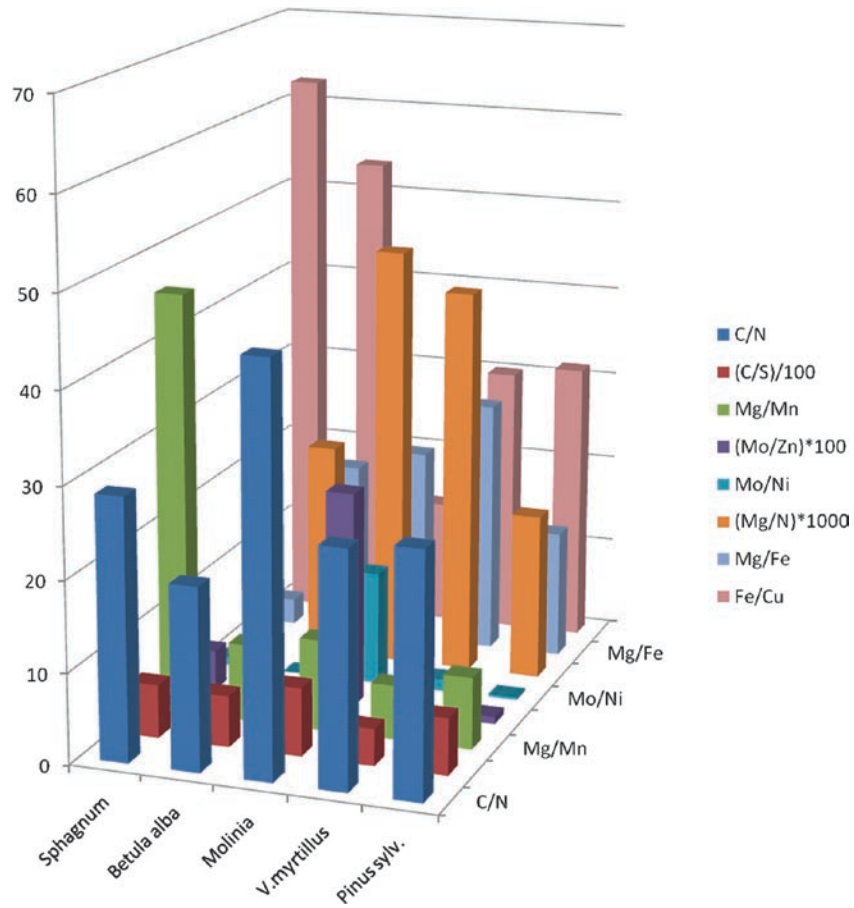


Fig. 2.26 Changes in ecological stoichiometry upon succession in a peatbog ecosystem (cp. Table 2.25)

ratios oscillate wildly, as does the Fe/Zn ratio which likewise denotes the relative importance of various metabolic interactions given the geochemical background (all this is about photoautotrophic metazoans, with no additional reason for variation). So, in a bog with lots of humic acids but only traces of nitrate, mobilization of those trace metals which are strongly coordinated to humics may depend on Cu supply rather than Mo abundances; hence, except for *Molinia*, the Mo/Cu ratio is small. The Mg/Fe ratio is kept rather constant after terrestrial plants took root in the area which implies the relationship of Fe-dependent plant enzymes and biochemical processes vs photosynthesis rates does not depend or change upon succession once vascular plants are involved while mosses need much more Fe. The trend Fe/Cu, however, is inverse: plants tend to require more Cu in dealing with soluble humic acids and possibly also with peat when the supporting medium gets less wet, with a subsequent decomposition pattern with respect to metal ions which differs from those in higher plants regardless of their role in succession. Probably this is linked to both the use of "exotic" sequestering agents such as proline, and the presence of (some necessity to absorb) substantial amounts of silicon with silicates being a possible additional storage site for certain metals (Mg, Ca, Fe, Ti) besides or in place of phytochelatins. Yet, even then there are no "smooth", steady changes in the trace element use patterns when humic substances change their identities and binding behaviors while sulfide gets scarce also, reducing the retentions of thiophilic heavy metals (except for those which form thioanions like V, Mo or W) and of Fe in the "ripening" bog soil with time and dryness.

Manganese also is low in other aquatic plants, whereas Mg/Mn is almost constant in the terrestrial

Table 2.25 Succession in a peat bog falling dry, typical plants and direction of succession from left to right. C/element and intermetal ratios undergo considerable changes not all of which follow simple patterns

Direction of suc-cession (from left to right)	*Sphagnum* spp.		*Betula alba*		*Molinia caerulea*		*Vaccinium myrtillus*		*Pinus sylvestris*
Element ratio	Mass	Stoich.	Mass	Stoich.	Mass	Stoich.	Mass	Stoich.	Mass
Mg/Mn	20.5	46.3	3.78	8.54	4.44	10.03	2.65	5.98	3.52
Mg/N	0.026	0.015	0.041	0.024	0.082	0.047	0.074	0.043	0.032
C/N	24.7	28.8	17.2	20.1	38.3	44.7	22.2	25.9	22.9
C/S	220	586	211	564	291.5	751	152	406	234
N/P	18.8	41.6	15.6	34.5	12.5	27.7	19.7	43.6	23.6
Mo (μg/kg)	2,340	–	56	–	16,600	–	400	–	620
Mo/Zn	0.065	0.044	2.7×10^{-4}	1.8×10^{-4}	0.36	0.24	0.020	0.013	0.012
Mo/Cu	0.35	0.23	0.020	0.013	1.98	1.31	0.082	0.054	0.14
Mo/Ni	1.38	0.84	0.056	0.034	20.8	12.6	>2	>1.3	0.44
Fe/Zn	10.1	11.6	0.63	0.73	2.22	2.56	6.5	7.5	2.23
Fe/Cu	55	62.5	47	53.4	12.1	13.8	26.5	30.1	27.6
Mg/Fe	1.24	2.85	8.55	19.6	9.58	22.0	12.3	28.3	6.21
Mg/Cu	68	177	400	1,044	116	303	326	851	171

C/S remains almost constant whereas N/P is steadily increasing with the terrestrial plants

plant during succession as expected from theory. C/S remains rather constant also. The Mo/Zn-, Mo/Cu- and Mo/Ni-ratios are used as first approximations for interactions of various oxidoreductases, with Mo used to reduce nitrate in (roots of) some plant dwelling on already rather solid ground, and Cu required for formation of polyphenols (flavinoids, gallic, caffeic, etc. acids, tannins).

2.3.4 A Corollary on Bioindication

All the data mentioned up to now were derived from analyses of plants or other organisms, and they are known to be linked to concentrations of these very elements in environmental compartments like soil. Thus, these concentrations in the Biological System of Elements can – and in fact often are – also exploited to get information on the composition of the environment, the approach being known as biomonitoring. However, it should be clear from the preceding work that the relationship between both sets of concentration levels (plant parts vs soil or groundwater or ambient water, respectively) is not straightforward particularly if there are sets of identical and thus deceptively simple-looking BCF values. We are thus poised to ask whether there could be something like an ideal bioindicator, doing no additional fractionation on its own besides of, possibly, some enrichment.

Theoretically speaking, the optimum bioindicator would be one in which, due to corresponding effective $E_L(L)$, influences of ligand sensitivity x and intrinsic bond stability c cancel out for all the considered items of biomonitoring, giving rise to identical BCFs. A chance for this arises from the correlation between c and x which at least holds for sets of ions of identical positive charges or, rather, oxidation states (the same effect could be achieved abiotically by introducing some polymer-fixed bidentate ligand into the test compartment and analyze the pattern of adsorbed metal ions, e.g. by stripping voltammetry). Such an organism can be dubbed "minimal" – primitive with respect to evolution – since it exists without selective transport systems for metal ions. This is no statement on other aspects of biological, histological or biochemical complexity or taxonomic status (it might be unicellular as well as metazoan, fungus, bacterium or animal), except perhaps for the properties of membrane interfaces (e.g., gills) for aquatic organisms. While such a minimal organism would behave as a perfect accumulation bioindicator, with internal element levels directly linked to those in the environment, this exactly would permit its survival only in an environment without larger changes of chemical parameters (in turn severely limiting its possible use as a bioindicator, too).

The intersection of the complex stability lines at $E_L(L) \approx -0.15$ V (Fig. 2.15) reveals a possible way into optimum bioindication, given the similarity of this value towards to that of many moderate-climate terrestrial plants (Table 2.7). But there can be some more general reasoning.

There are certain typical abundance distributions of trace metals in either animals (Fe ≈ Zn > Zr (inactive) > Cu > Cd (probably essential for growth) > Mn > Cr (role uncertain) > Co > V > Mo; Kaim and Schwederski 1993) or plants (e.g. leaves of *Vaccinium myrtillus*; Ca > Mg > Si > Mn > Al (inactive) > Ba (inactive) > Zn > Cu > Ni; Markert 1996); for an unspecified pool of plant species and parts bioconcentration factors are given (Kabata-Pendias and Pendias 1984), prompting the question whether these distributions could arise simply by selective uptake from soil-, fresh- or seawaters by rules of coordination chemistry. If so, such an organism would be an ideal biomonitor as its BCF values could be readily predicted from just a couple of measured values. However, the (at least) recent distribution of essential trace metals in environment requires selective sequestering and transport in organisms and directly around them while "harmless" non-essential elements tend to behave in the above manner indeed. Figure 2.16 shows how and why maxima of BCF in green plants are linked to certain (not extreme) values of x_{2d} in, e.g. neutral oxidizing soils by soil- or (organic) limnochemistry providing and interconverting ligands.

From theory, such an optimal bioindicator would be an aquatic organism in which contributions of ligand sensitivity and intrinsic bond stability will cancel for all the metal ions under scrutiny, giving unbiased BCF regardless of the element and its chemical properties. Since c and x are correlated for identical oxidation states (+II or +III) this is conceivable, but one might as well abandon the biomass altogether for using some polymer endowed with (bidentate) ligands of appropriate $E_L(L)$ value, exposing the polymer to the environmental sample and then do stripping voltammetry. The biological system would be some "minimal" or – in terms of evolution – "primitive" biomonitor organism insofar as it lacks means of any selective ion uptake and transport, to some extent being even more primitive (less selective) than the early polymer of chemical evolution phase 3 discussed below (Chapter 4). Taxonomical position, biochemical and histological complexity of such (hypothetical) minimal organisms do not at all matter (i.e., they also may be rather "advanced", e.g. trees or vertebrates) unless the undiscriminate binding behavior of the metal ions taken from environment would correspond to several different $E_L(L)$ values. Then transfer across membranes and the corresponding binding patterns would give rise to selective uptake. This hypothetical minimal organism is (rather, would be) an ideal bioindicator exactly because its internal metabolom is exactly related to environmental concentrations which, however, means that any real organism would survive only if there are no major changes in concentrations and ratios thereof around (in turn putting considerable practical limitations to biomonitor/bioindicator applications).

Take two examples of organisms often used in biomonitoring, namely *Daphnia magna* (water flea, a small crustacean) and some higher plants. Notably, either abundance series Fe ≈ Zn > Zr > Cu > Cd > Mn > Cr > Co > V > Mo or Ca > Si > Mn > Al > Ba > Cu markedly differs from the Irving–Williams series, even that extended to trivalent ions like Al, Fe(III), but in fact we have to consider the BCF values rather than absolute levels. Looking to the different parts of Scots pine *Pinus sylvestris* once again, we find V ≈ Fe < Ti < Al < Cr << Cu ≈ Co < Ni < Mn (young needles) or Fe < Ti = Cr < Al < V << Cu ≈ Co < Ni < Mn (bark), that is, almost inverting the Irving–Williams series for the last four ions, with a particular status for Fe, possibly trivalent. This strange result, corroborated for the microroots (that is, V < Cr < Ti = Co < Ni < Al < Cu ≈ Fe < Mn) directly interacting with soil solution, can be simply explained: there are chelating ligands in the soil also, retaining metal ions to some extent when they strongly bind them (like Fe(III), Cu(II) or Co). Our work (Fränzle and Noack, 2008) suggests Leptothrix ochracea (fig. 2.12) to be next to an ideal aquatic biomonitor for heavy metals, requiring O_2 and (about 1 mg/L) Fe_{2+} (active monitoring), yet is difficult to cultivate.

Chapter 3
A Causal Model of Biochemical Essentiality

3.1 Influence of Intrinsic Bonding Stability and Ligand Sensitivity on the Biocatalytic Properties of Metal Ions

So it might (have proven to) be useful during evolution to increase the number of essential components, while the separation of the ancestors of animals and higher plants took place only after formation of metazoans, and even though the number of possibly essential (chemically suitable and sufficiently abundant) elements is limited to about 40 at best, the rule of three functions produces another limit as it also holds for molecular "vitamins", of course. The result will be some optimum function for the number of essential elements vs. histological complexity (and animals tend to use more different elements than plants, fungi or bacteria) which accordingly tends to be a smooth, steady one. Presumably this is why earthly organisms did not evolve into metazoans for most of the time of biological evolution, even much after oxygen did accumulate in the atmosphere. If so, the group of organisms forming the original basis of the Biological System of Elements – vascular plants and some mosses – had to be considered atypical, however. On the other hand, as the results reported here come directly from chemical physics pertinent to any kind of reproducing system, they will apply to any kind of living being, not just photoautotrophic metazoans. As pointed out before, plants are autotrophs and hence are more capable to interconvert quite different speciation forms of some essential element, with the number of essential elements in higher plants being about 16 (cp. Table 2.1). Autotrophs by definition convert these species taken up from the environment (e.g. soil, atmosphere) into other speciation forms far beyond the possibilities of any heterotrophic organism, which implies, using the relationship

$$F = K–P + 2 \tag{3.1}$$

that the number of "genuine" components is not larger than that of the elements. However, it will not be smaller either because none of H, O, S or C will be inevitably coupled to "relevant" speciation forms of all the other elements in biochemistry. For example, N might occur as an oxoanion (preferably nitrate) but can readily be converted into storage and transport species with both N-C- and N-H-bonds (ammonia, urea, amino acids, purines) some of which do not contain any oxygen. Allowing for some minimum of degrees of freedom, the number of phases P is $\mathbf{P \leq 17}$, defining the upper limit of histological complexity in vascular plants. Hence, as noted before, it is the very metabolic feature of autotrophy which keeps plants from becoming as complicated and diversified in terms of histology as higher animals can and tend to be. The phenomenon that autotrophs command usually less different essential elements – and hardly any in larger amounts – than heterotrophs (cp. Table 2.1 above) which can be accounted for by the above lines of stability reasoning is the more noteworthy as autotrophy is more demanding in terms of chemical synthesis tasks than heterotrophic metabolism which makes use of rather sophisticated educts and externally induced chemical energy, chiral information etc. and hence autotrophy might be assumed to require larger amounts of more different catalysts. This is obviously precluded by the physicochemical limitations discussed before.

Hitherto certain metals which are known to be essential were discussed with respect to their matter flow rates, sources, sinks and autocatalytic orders, using Eq. 2.4 as a kind of background information to

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determine or estimate complex stabilities and in turn partition of the element. As c and x, accordingly, are parameters which influence partition and the chance to use some element altogether, it may be anticipated that only some combination(-range)s of x and c can permit efficient autocatalysis and thus biocatalytic essentiality. Therefore, just try to do some mapping of biochemical properties in the c/x plane for the distribution of essential elements. A simple look on Fig. 3.1 will show that there is some relationship between x and c for divalent ions and another for trivalent ions even though x and c can plausibly be identified with π- and σ-bonding properties of a metal ion, respectively (see the large $E_L(L)$ of strong π-acceptor ligands like CO, ethyne, phosphines or phosphites and the decrease of x from Cu(II) towards Zn(II)). With the present knowledge on the spectra and distributions of essential elements and on other effects of chemical elements such a c/x/ "function" plot (Figs. 3.1 and 3.2) may reveal common features of the essential metals with respect to metal ion-protein interactions.

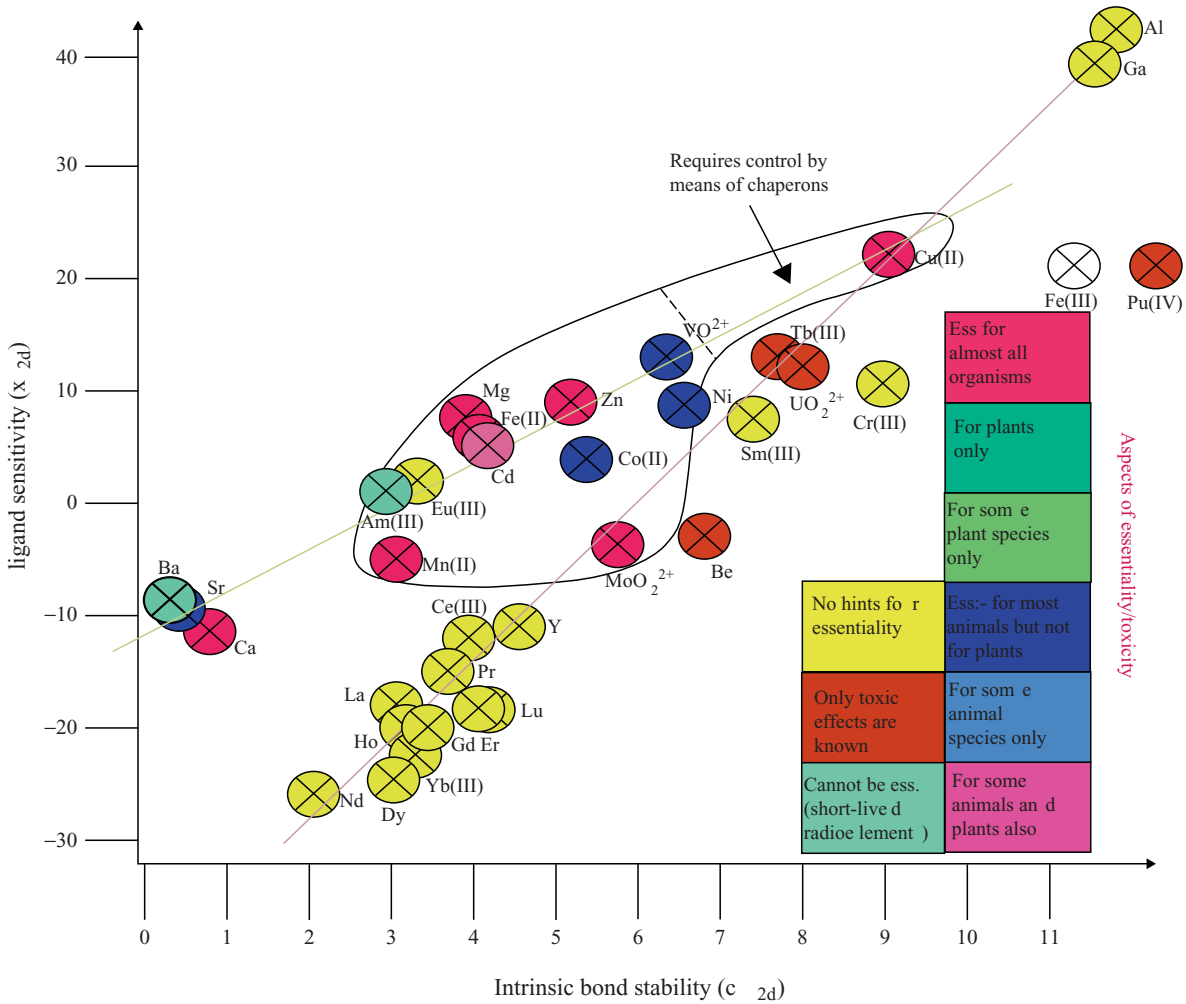

Fig. 3.1 c_{2d}- vs. x_{2d} parameters of metal ions and essentiality. The exact values of c and x correspond to the crossing point of the lines inside the dot while the shadowing of this dot denotes existence and possibly range of essentiality (if there is any). While Fe(III) is absorbed by bacteria, it gets its biological functions mostly (exception: haem peroxidases) only after reduction to Fe(II); hence the symbol for Fe(III) is not colored at all. The actinoid elements U, Pu and Am are highly toxic but might be regarded either as radiopoisons or as chemical noxes. Both for uranium ($^{234;235;238}$U) and plutonium ($^{242;244}$Pu) radioactive damages become negligible when compared to hazards by purely chemical modes of actions in the nuclides of longest half-lives (for those nuclides mentioned here: 10^5 a << $\tau_{1/2}$ < 4.5 × 10^9 a) while there is no such isotope for americium (Z = 95; $T_{1/2}$ = 7,400 a for ^{243}Am, much less for all other isotopes); thus the different shadowing of the dots. The line which connects the essential divalent elements Sr, Ca through Zn, Cu follows a regression equation (eq. 3.2)

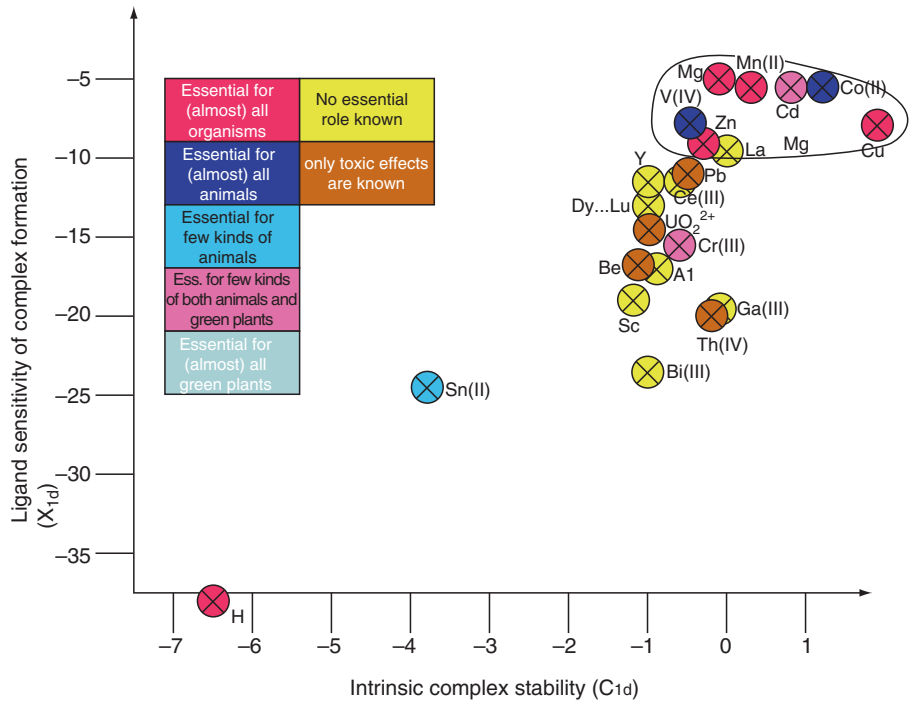

Fig. 3.2 Essentiality and other kinds of biological function vs. c- and x-parameters given monodentate complexation. Given the range of values feasible with monodentate coordination, all the essential elements except of Cr(III) are distinguished by relatively high intrinsic binding strengths combined with a low sensitivity. With monodentate binding modes rather limiting complex stabilities, it is likely that this combination of c and x is required to effect any activation of substrates by metal ions if chelate formation is avoided. The physiological function of chromium in enzymes is unsettled – if there is any indeed; it does rather inhibit plant growth (Williams and Frausto Da Silva 1996) although it is accumulated by several plant species like Shepard's purse (*Capsella bursa-pastoris*) (Kaim and Schwederski 1993). To our present knowledge, vanadium is not essential for green plants (unlike for certain fungi), with its typical leaf or fruit concentrations ranging in between 100 and 700 μg/kg (ppb; see Kabata-Pendias and Pendias 1984; Markert 1996) which is considerably more than in animals (few ppb) which actually need it, but there is an interesting exception: far higher V levels (about 50 mg/kg) are seen in plants like sunflower *Helianthus annuus* (Emsley 2001) which produce large amounts of linolenic acid, whether these phenomena are related or not. H (lower left of the diagram) is an exceptional case for its covalent mode of binding and total lack of π-bond interactions; the values given in this c/x plane were calculated from aqueous acidities of oxoacids (nitric, perchloric, acetic, trifluoroacetic, triflic etc., also anions such as HSO_4^-, $H_2PO_4^-$) and $E_L(L)$ of the corresponding ligand anions. Thallium, for which Tl(I) probably is the only biologically relevant oxidation state, is the only element where c_{1d}- and x_{1d}- (or any such) values depend on the kind of donor atom, with generally weaker bonding in O donors such as hydroxide, nitrate or acetate (cp. Table 2.3)

Within the c/x plot for bidentate complexation there is a very constrained region containing all the classical (and some "exotic" or ultratrace) essential metal ions other than Cu, which exert biocatalytic functions in the center. The heavier alkaline earths Ca. Sr (for corals and foraminifers) and Ba (in desmides) are essential elements but hardly for purposes of biochemical catalysis, that is, substrate binding measured by c and x does not matter for heavy alkaline earth essentiality.

There are also cases when one given function can be promoted by different metal ions; then, as a rule, these are very close to each other in the c/x diagram, e.g. Zn, Co(II) and Cd ($c \approx 5$; $x \approx 6.5 \pm 2.5$) for bidentate ligands) in carboanhydrases of identical plants (Price and Morel 1990; Strasdeit 2001) with presumably bidentate binding of HCO_3^- (Kaim and Schwederski 1993; Lipscomb 1982; Christianson and Fierke 1996; Kimura 2001). Carboxamides (peptides, urea), carbamidines and nitriles undergo hydrolysis catalyzed by Zn, Co(II) (bacteria, animals) or Ni(II) (in plants, e.g. jack-bean urease) (Frausto Da Silva and Williams 2001), that is, $c \approx 6$; $x \approx 7.5$. Thus conditions for (bio-)catalysis of a given reaction correspond to some rather small region in the c/x plane in either case, with a straight line which connects the essential divalent

cations, providing **limiting conditions for uses in bioinorganic chemistry**. This "window of essentiality" comprises Mg, V(IV), divalent Mn, Fe, Co, Ni, Cu, Zn, Cd, notwithstanding their grossly differing abundances in metalloproteins. But abundance does not matter in defining properties coming from or being related to c and x parameters which are required in activating substrates.

3.2 Complex Stability in Relation to Other Bioorganic Parameters

There are many examples from bioinorganic or biomimetic chemistry when simple model complexes accomplish the same catalytic functions like a metalloprotein they are meant to mimic, even though coordination environment (ligand sphere) of the central ion and thus spectra and redox potentials may differ considerably from those of the metalloprotein. If embedded into lipid membranes or displaying many unpolar amino acid residues around the metal site, the latter affords a local surrounding rather different from water with respect to dielectric constant, (non-)periodicity of solvation potentials etc., the former causing changes in $-\log k_{diss}$ also. There are even cases where simple components self-organize spontaneously to yield some system which fulfils the wanted function, the most prominent example being the self-assembly of a nitrogenase functional model (Schrauzer 1975) from MoO_4^{2-}, Fe(II) and some thiolate, which like nitrogenase only needs an additional reductant (usually BH_4^-, but the pyrrhotite/H_2S system will also do) to reduce all dinitrogen and other nitrogenase substrates like CN^-, ethyne, isocyanides affording NH_3 or C_2H_4 and corresponding by-products such as CH_4, C_2H_6 or secondary amines (R-NC {nitrogenase} → R-NH-CH_3) (Eady 2003). Another important example of self-organization of some biomimetic catalyst system is seen in iron-sulfur-heterometal clusters (cp. Mansy and Cowan 2004 for their biochemical roles), and metal ions use to behave as templates during formation of coordination polyhedral in metalloproteins (Liu and Xu 2002).

The actual activity of other nitrogenase (purported) models towards both uptake and reduction of gaseous N_2 depends on the redox potentials of Mo phosphine complexes and thus the electrochemical ligand para-meters of the coligands (Chatt et al. 1980a), and so do the optical spectra, while just 1–2 of the involved ligands (XY; X = N, CH or C^-, Y = N, CH or RN^+) behave as substrates. When there is some biomimetic catalytic reaction promoted by the same central in as in biochemistry, like in the cases above and many others, this gives proof that coligand effects are minor to control by c and x parameters (given, of course, that the reaction occurs at comparable sites indeed).

Considering the values given e.g. by Williams, complex formation constants of metal ions in or to metalloproteins agree with those of complexes which contain simple oligodentate chelators which comes to no surprise when donor sites are similar or identical in kind and number in simple chelates and in proteins. Thus, there are no specific properties of protein polymer backbones which would distinguish them from simple complexes, except for the "chelate effect" which is included in this model as a dependence of c from denticity ($c_{1d} < c_{2d} < c_{3d} \ldots$). This chelate effect is relevant only if five- or six-membered rings are formed by chelation (Tobe 1976; Jordan 1994). In fact, Fig. 3.1 is based on this similarity between metalloprotein metal ion retention and the complex formation constants of bidentate ligands; in addition, the equilibria for higher complexation give rise for labile ligands to be replaced by the substrate(s). The "entatic principle" (Vallee and Williams 1968) refers to spectroscopic peculiarities of metal proteins, especially such which contain Cu(II) in their centers, which disappear once the substrate (or similar yet irreversibly binding other ligands) has been bound, filling up the coordination polyhedron. If complexation is fluxional, that is, bi- and tri- or tetradentate complexation of the metal ion by the apoprotein are similarly stable in aqueous environment, which means $\Sigma E_L(L)/n$ (n = 2 or 3 or 4) is close to the critical value given in Tables 2.5 and 2.6 this entatic effect is assumed to assist substrate binding. Often the entatic effect is attributed to some distortion of coordination polyhedra which alters the energies of metal-centered orbitals and thus redox potentials and complex stabilities also (cp. the Angular Overlap Model; Mingos 1984), producing complexes with redox potentials which deviate from the additional increments by Lever and others. For e.g. Fe or Cu containing metalloproteins, both kinds of change can be observed, concerning

both the dd band wavelengths and the redox potentials (the data for $Ru^{II/III}$ can be adapted to other metal ions by linear regression).

Generally speaking, complex formation constants estimated using Eq. 2.10 do well agree with those from other sources and methods (Vallee and Williams 1968; Williams and Frausto Da Silva 1996) on metal ion binding equilibria in proteins. Thus both stationary states (equilibria) and enzyme catalysis of reactions can be addressed. Hence for this first approach c- and x-values for a metal ion inside a protein should be identical to those for the aquaion, except if there are macrocyclic coligands such as porphyrines. Binding of some sixth and thus terminal non-water ligand to a ML_5 fragment was investigated for some Co(III)- and Rh(III) complexes providing values of $c_{1d(term)}$ and $x_{1d(term)}$ (cp. Table 2.3). There is no chance for direct comparison as $(Co(H_2O)_6)^{3+}$ is a very strong oxidant (Cotton and Wilkinson 1981; Mizerski 1997) precluding its combination with most ligands while avoiding metal reduction and thus $c_{1d(prim)}$ and $x_{1d(prim)}$ cannot be determined for Co(III). The very existence of phenomena like the *trans*-effect does show that *kinetics* of ligand exchange do, yet, depend sensitively on the ligands already bound, e.g. CO.

It is obvious from Fig. 3.1 that some strongly toxic elements, like Be (Kaim and Schwederski 1993), Tb (Emsley 2001) or uranium (as UO_2^{2+}) do resemble essential metals like Mn, Mo or V with respect to x_{2d} but are distinguished from these by considerably higher c values, that is, stronger coordination, whereas most (other than Eu, Sm, Tb) REE trications have c_{2d} values close to those of Mg, Mn(II) or Fe(II) but the corresponding x values are much lower. This suggests that too strong binding of metal ions to biomass is more "dangerous" than highly negative ligand sensitivities: while the former would be destructive for even "plain" catalysis in accord with Sabatier's principle, SNA demands some lability in autocatalytic systems beyond this, and the latter just reduces the set of key reactions possibly catalyzed by one given metal ion below the lower limit of 3 required to sustain catalytic biology. Elements like these – the situation is similar with Cr(III) – are thus not (sufficiently) versatile but not too toxic either, while there will be misallocation and thus functional inhibition of proteins anyway if both c and x are very high (Al, Ga, Pu(IV); Ga^{3+} acts as a cytostatic agent replacing Fe from many metalloenzyme centers). Even then ligand sites with profoundly negative $E_L(L)$ values such as phosphate esters will be effectively blocked (Guo and Sadler, 1999). If, however, some highly negative x_{2d}-value combine with moderate to low c_{2d} values (which is possible with oxidation states ≥ + III only, not with dications, e.g. in Nd, Dy, Ti and its heavier homologues Zr and Hf), the outcome will be selective inhibition rather, being relevant in toxicology or cell budding in some cases (for example, when the phosphate "switch" is affected, attacking serine kinase products) but usually no systemic damages will be seen in most of very diverse organisms.

Equation 3.2, in addition, provides an orientation value (which is not to be taken as a lower limit!) for a low $E_L(L)_{eff}$ of biomass, assuming that the line through the c/x plotting is due to some principal site of metal-bioligand interaction of appropriate electrochemical ligand parameter; as it corresponds to the entire set of essential biocatalytic metals it is likely that corresponding ligand sites are involved in metal uptake rather than specific modes or items of catalysis as there is no single reaction which would be promoted by all or even most of the essential metal ions. In some way the observed set of essential ions then reflects the properties of the above principal site which thus may be (tentatively) identified. This average value is 1/(−3.085) = −0.323 V, close to values for phosphate esters which are in the center of energy metabolism, reductive activation of crucial non-metals (ATP being used in both sulfate reductase and N_2 activation), bringing about uptake (though there are often insoluble phosphates) and functionalization of the elements along the line like Mg, Mn, Cu or Zn and sometimes V or Cd (the speculation is tempting to consider which set of metal ions would be essential if other oxoanions rather than P-centered ones – say sulfonates – or even halide or halometallate ions would be used in biology as principal leaving groups. Then, conceivably, another line would hold, probably in favor of trivalent cations including Al and REEs, besides Cu and Mo).

Whereas the metal ions in the metalloproteins are not equally strongly bound, they all interact effectively with both biomass and possible substrates to catalyze various reactions (>3 each); at −0.32 V, the ranking of complex formation constants of essential metal ions would be Mg < V(IV) ≈ Cu(II) < Fe(II) <

Zn < Ni < Co(II) ≈ Ca < Mn(II) << Mo(VI) (last column in Table 3.1 below), that is, with grave deviations from the Irving-Williams series as $E_L(L)$ is so low. Except for Mg, Ca, and Sr in seawater metal ions would not be reliably retained by biomass in these conditions. Comparing the bi-/tetradentate binding equilibria for many of these metals (Table 2.5), it is obvious that the multitude of possibly nearby ligand sites in an average metalloprotein would not cause "stochastic" binding to random donor sites or "saturation" of the ligand sphere. Interestingly, no biomass sample was identified up to now the $E_L(L)_{eff}$ of which would be identical to or even lower than the above −0.323 V.

Asking for a chemical "meaning" of this value, it is found to match the electrochemical ligand parameters of nucleic acids or of low-molecular phosphorylated compounds, as noted above (also cp. Table 2.2). Accordingly the present set of metal ion biocatalysts might have been circumscribed in the very moment oligophosphates got their multiple roles in biochemistry (or late prebiotic chemistry? Chapter 4). In a hypothetical biochemistry based exclusively on amino acids, including peptide replicators besides heterocycles for catalysis and metal mobilization, that is, average $E_L(L)$ somewhere in between −0.05 and +0.3 V, only a few of these metals would keep about their terrestrial biological roles, possibly Mg, Zn or Co, but excluding others such as Mn or Cu in favor of Cd, Ni or Cr(III) (no or negative inclination of the respective line). Complex formation constants for $E_L(L) = -0.323$ V and bidentate binding are given in Table 3.1.

Table 3.1 Complex formation constants for $E_L(L) = -0.323$ V, representing binding into metalloproteins. Data are given for essential (biocatalytic) and some other, both essential and undefined elements

Metal	c_{2d}	x_{2d}	$-\log k_{diss}$
Mg	3.94	8.24	1.28
Ca	0.73	−10.98	4.28
Sr	0.55	−8.93	3.43
Ba	0.45	−8.02	3.81
VO^{2+}	(6.44)	(14.17)	1.86
Cr(III)	(8.90)	(13.90)	4.41
Mn(II)	3.01	−5.32	4.73
Fe(II)	4.20	6.05	2.25
Co(II)	5.48	3.93	4.21
Ni	6.65	8.86	3.79
Cu	9.04	21.84	1.99
Zn	5.15	8.69	3.34
Cd	4.3	5.7	2.46
Mo(VI)	5.74	−3.78	6.96

It is possible to plot relationships between the c and x parameters of metal ions for different hapticities and essentiality for various organisms simply without making any statements or assumptions concerning biocatalytic functions – whether if there are any or concerning catalysis of processes of whatever kind c and x are plotted vs. each other, with essentiality being some third property (Fig. 3.1), of course regarding Sabatier's rule on relative strengths of "optimal" catalyst/substrate interactions (being neither too strong nor too weak), which leads one to assume that essential elements will gather in certain regions of such c/x plots.

There is a similar line for trivalent cations but not for tetravalent ones while also some essential divalent ions are located somewhat off this line (Mn, Mg, Co). Highly toxic dications, however (Be^{2+}, UO_2^{2+}), are located far to the right of this line. It cannot yet be said whether higher oxidation states of the same metal ions (Fe, Mn, V) in redox enzymes such as sulfite- or aldehyde oxidases (Mo), cytochrome peroxidases (Fe) do "indeed" conform to the parameter region depicted in Fig. 3.1. In some cases, like for vanadium haloperoxidases, it is known that these enzymes do coordinate substrates in "normal" oxidation (i.e., V(IV)) and then the central cation gets oxidized without there ever being direct contact between this cation and the oxidant (outer sphere oxidation, perhaps mediated by H bridging), e.g. with O_2 or H_2O_2.

Within the c/x plot for bidentate complexation there is a very constrained region containing all the classical (and some "exotic" or ultratrace) essential metal ions other than Cu, which exert biocatalytic functions in the center. The heavier alkaline earths Ca, Sr (for corals and foraminifers) and Ba (in desmides) are essential but hardly for purposes of catalysis, that is, substrate binding measured by c and x does not matter for heavy alkaline earth essentiality.

There are also cases when one given function can be promoted by different metal ions; then, as a rule, these are very close to each other in the c/x diagram, e.g. Zn, Co(II) and Cd ($c \approx 5$; $x \approx 6.5 \pm 2.5$ for bidentate

ligands) in carboanhydrases of identical plants (Price and Morel 1990; Strasdeit 2001) with presumably bidentate binding of HCO_3^- (Kaim and Schwederski 1993; Lipscomb 1982; Christianson and Fierke 1996; Kimura 2001). Carboxamides (peptides, urea), carbamidines and nitriles undergo hydrolysis catalyzed by Zn, Co(II) (bacteria, animals) or Ni(II) (in plants, e.g. jack-bean urease) (Frausto Da Silva and Williams 2001), that is, $c \approx 6$; $x \approx 7.5$.

$$x_{2d} = 3.545 \times (c_{2d} - 3.085) \tag{3.2}$$

Formally speaking, the distribution of "biometals" inside the c/x-plot (Fig. 3.1) is similar to that obtained by Williams for correlation with "borderline" behaviour of polarizability when chelating, bidentate ligands are concerned. As for monodentate ligands, biochemically active metals rather gather in the uppermost right part of the c/x plot (except the data for Tl(I); Fig. 3.2), with both c and x unlike the values observed for bidentate chelators. This rather contrived, non-statistical distribution can be explained in terms of dynamic rather than static ligand-metal-interactions, that is, of catalytic versatility: we then do no longer consider retention of an unchanged ligand but some cycle which changes the identity and thus binding properties of a substrate which is processed in a periodic fashion, with the catalytic cycle being closed if some conditions are fulfilled.

Among the substrates of "principal" biochemical transformations there are few distinguished by "extreme" $E_L(L)$ values, namely OH^-, thiolates and CO, restricting the possible functions of metal ions with very low x_{1d} values (these ligands are monodentate, and there are very few values of $x_{1d} > 0$) so far as to render them irrelevant in catalytic biochemistry. As a rule, disulfides formed by thiolate oxidation themselves undergo cleavage by oxidative addition to a variety of metal centers which is implicated in e.g. Hg or organogold toxic effects (Kaim and Schwederski 1993; Cotton and Wilkinson 1981); nevertheless this versatile reaction apparently is not used by living beings, with bis-thiolate/disulfide redox cycles, e.g. in glutathione, be organized rather without directly involving metal ions. One might assume this may be compensated by exceptionally high (or low) values of c, but these are rare and restricted to rare elements. Finally it is conceivable to change (both c and) x by redox processes at the metal center to widen the spectrum of catalyzable processes but regardless of the chance to use ATP for coupled transfer of electrons and protons to some substrate the redox potentials cannot be decreased indefinitely within (aqueous or at least (lipids!) protic) biomass. What can be gained from this may be estimated by first considering the most strongly reducing compounds or ions produced by living beings in other than photochemical processes (i.e., excluding CO_2^- and the hydrated electron) and then projecting these potential/pH values to Pourbaix diagrams of metals (or their respective carbonyl complexes) and non-metals for a stability estimate, expecting e.g. Co(I) and Ni(I), probably also Mo(III) and low oxidation states of V, in certain kinds of biomass. So what is obtained indeed? Recently, remarkable bioproducts concerning ε_h (that is, requiring thorough reductants for formation) were identified, including

1. PH_3 (cp. Jenkins et al. 2000; Eismann et al. 1997 for pathways of phosphine biosynthesis), selenocystein, AsH_3 (produced by various pseudomonads and *Alcaligenes* bacteria) and eventually even stannane SnH_4 (released besides some methyltin hydrides during anaerobic degradation of marine macroalga *Enteromorpha* spp. (Donard and Weber 1988)). While selenocysteine even ranks among the proteinogenic amino acids, there is no evidence for hydrogenation of either Sb[1], Se or Te (Thayer 1995) to produce the binary EH_n compounds (Te-H bonds are not produced by any known organism, rather Te colloids are deposited (Woolfolk and Whiteley 1962)) whereas
2. Clostridia and some archaea succeed in releasing H_2 from organic matter, therefore often using W(+Se)-dependant enzymes, reductions of W and Se being considerably more difficult than those of Mo and S, respectively.

From our present knowledge, stannane SnH_4 is likely to be both about the strongest reductant and the hydride with the weakest H-E bonds ($\varepsilon_{H-SnH3} = 2.75$ eV) which can be produced by biological systems

[1] See the controversy about inferred formation of and intoxication by stibane SbH_3 purportedly produced by fungi in moist matraces (Emsley 2001; Thayer 1995) which was related to cot death syndrome (SIDS): in fact, SbH_3 simply does not form in these circumstances.

directly. Species in a Pourbaix diagram of whatever element which are more reducing than SnH_4 (formal potential: −1,480 mV at pH 7) hence (most probably) need not be considered for biochemistry or biocatalysis. The weakness of Sn-H bonds precludes formation of either SnH_4 or of organotin hydrides R_xSnH_{4-x} if they were to be formed by hydrogenolysis of Sn-C bonds by e.g. formate unlike it may happen with P or As: both PH_3 and AsH_3 can be prepared by heating the corresponding tetraarylpnictonium salts with formic acid if catalyzed by transition metal ions (own observations of this author). Accordingly, SnH_4 formation is likely to be an endothermic process requiring ATP, e.g. for formally replacing oligophosphates (Sn/P heteropolyphosphates) formed from oxostannates and ATP with hydride ions. Thus, only triple points around which redox catalysis may be organized by metal or non-metal (e.g. Se) catalysis above the SnH_4/Sn^{2+} couple are relevant.

Apart from elements such as V, Mo or Co where multiple oxidation states are accessible within this limit (+mixed-valence compounds, hetero- and homopolyacids), ligands like cyanide, CO or NO which also occur in enzymes (e.g. hydrogenases) will also stabilize low oxidation states of other metals (here: Fe, Ni) owing to their high $E_L(L)$ values, like oximes or porphyrines also do (nitriles or pyridines also could do so but not hydroxamate siderophores). Organometal biochemistry at Co or Ni centers can occur and induce secondary biomethylation to yield either methylelement compounds, acetate (rather like Reppe's and Collman's reagents) or CH_4 by formal carbanion transfer only if such stabilization had occurred (but not to an "extreme" extent; for example, $(Ni(CO)_4)$ or $(Co(CN)(CO)_4)$ are too difficult to oxidize for catalyzing such reactions, unlike their phosphine derivatives). While this class of redox biocatalysts is rather constrained and thus homogeneous, there is larger "scatter" in properties and variation of co-ligands (not only porphyrines, imidazols and CO) in other redox enzymes containing Cu, $Fe^{III/IV}$ (cytochrome-P_{450} and related enzymes), V or Mo in their active centers differ considerably with respect to both the substrates and the reaction mechanisms (Kaim and Schwederski 1993; Frausto Da Silva and Williams 2001). In order to avoid misallocation of the strongly complex-forming Cu^{2+} ion to a multitude of proteins, there exist specific transport and regulation proteins chaperons (Williams and Frausto Da Silva 1996; Tottey et al. 2005; Rosenzweig 2001). By this way of control Cu becomes useful even though its properties differ from those of all other metals which comprise the "window of essentiality". Yet, from my point of view, it would be premature or too far-fetched given our present knowledge to infer either that Cu became essential only after molecular oxygen enrichment in the Precambrian environment and oxidative dissolutions of cuprite/chalcopyrite (Cu_2S and $CuFeS_2$, respectively) sulfides (Williams and Frausto Da Silva 1996), or – being the other extreme position – invoke a role for Cu(II) already in prebiotic chemistry from the fact that it can catalyze amino acid polycondensation to yield peptides (Le Son et al. 1998), with some autocatalysis by Cu(II) complexes of the primarily formed dipeptides. Although there are solid arguments that the event of biogenesis not only come along but was **identical** with far-spread and multiple use of metal ions in early replicating structures (whatever their chemical identities – peptides, modified clays etc.) (see Chapter 4), it cannot be said for Cu (or any other element other than C, N, H, O, S) which was the exact moment of its becoming essential.

Fe poses less of a problem although it in turn became less available with onset of oxidation by Fe_2O_3 precipitation (yet, there is replenishment of Fe(II) by both volcano eruptions (olivine) and aquatic photoreduction), with c_{2d}– and x_{2d} parameters of Cu(II) and Fe(III) being rather similar to each other.

On the other hand, there are highly abundant metals like Al and Ti which yet are not essential for any known organisms (not even forming supporting structures like with Sr or Si in certain cases) but which are both far remote from the above "window of essentiality" referring primarily to metal cations/complexes involved in biocatalysis (Ti(III) and – by extrapolation – Ti(IV) are located way below the "window", having $x_{2d} < -60$ while Al(III) is placed much above this range of values at $x_{2d} = +41$ and $c_{2d} \approx 12$). While elements like Al, Ti or Zr can indeed catalyze single of the principal biochemical reactions, e.g. hydride transfer among aldehydes to fuse them to esters or reduce other compounds such as C=N– or other C = element double bonds (Sykes 1977), one should note that fairly diverse reactions like CO_2 hydration, amide or ester hydrolyses, hydroxylation of organic substrates etc. are promoted each by one or some members of this "window of essentiality", suggesting the "catalytic scope" of ions having thus "deviating" values of c_{2d} and/or x_{2d} are not capable of doing bioca-

talysis in a way sufficiently diverse to fulfill the three-functions-role. In metazoans (except of green plants), one important essential transition element is almost confined to one kind of cell organelles: Mn in mitochondria. Concerning the non-essential elements, abundances of europium Eu are less tightly correlated with those of the other REEs including yttrium than those among each other (see above, Tables 2.19 and 2.20, and Markert 1996).

Moreover, Eu is placed in the c/x (bidentate) map considerably far off the other REEs except of Sm and Tb, the latter showing toxicological peculiarities also (Emsley 2001), with Eu(II) placed next to Ba, Sr, unlike other REE^{3+} or Ce(IV). Taking the existence of some contrived area in the c/x plane to be an established precondition for essentiality now (except, perhaps for Ca and Cu), one is caused to ask how the ligand properties of either substrates or products of principal biochemical transformations control the set of possibly or actually biocatalytic elements? It was outlined before that

1) There is some relationship to binding properties of P-oxospecies.
2) Biocatalytic versatility as according to the "three-functions-rule" pervades biochemistry even though only about one-third of enzymes contain any metal ions.
3) Apparently the number and kind of essential elements in producers is controlled and eventually limited by their "grazers", so there is some kind of ecological control.

which, taken as a whole, give some hint on the answer to this above question. Yet a final answer must be postponed to later work.

Although relating essentiality to the c/x plane for bidentate interaction with ligands was not a tacit assumption and is corroborated by the complex formation constants for most metalloproteins, one still needs to have a control, by looking on a possibly different or more "scattered" distribution of the essential elements in maps for different denticities. This is given in Fig. 3.2 considering monodentate behavior: essential elements now gather also albeit in some other region of the diagram, to the upper right rather than somewhere in the center. Monodentate behavior does occur e.g. in phosphatases and P-transferases which can contain and apply each of Zn, Mg, Mn(II) or (*high-spin*) Fe(II). So see some "window of essentiality" for these simple ligands again; similar plots for tri-, tetra … dentate ligand binding modes cannot yet be prepared for lack of data.

Figures 3.1 and 3.2 show that there are rather contrived areas in the c/x plane "permitting" essentiality regardless of either mono- or bidentate ligand binding concerning biologically plausible oxidation states of the said elements. When bimolecular processes are catalyzed, be it hydrolysis or linking to phosphates (kinases) or joining some other molecules (peptide ligases), either of the two substrate molecules might interact with the metal center or it just polarizes some assembly of amino acid side chains which promote the reaction by providing H bonds, thus adjusting the reactive centers of the substrates or donating (Asp, Glu) or abstracting (Lys, His, Arg) protons thereafter.

As noted before, ligand sensitivities of biocatalytically essential elements are rather close to 0 or slightly positive regardless of denticities of substrates. The other case of changing denticities between product and substrate, obeying the catalysis condition, corresponds to

$$\left\{x_{2d} \times E_L(L)_{prod} + c_{2d}\right\} < \left\{x_{1d} \times E_L(L)_{subst} + c_{1d}\right\} \quad (3.4)$$

(for change from monodentate substrates to bidentate products, e.g. in peptide hydrolysis)

or

$$\left\{x_{1d} \times E_L(L)_{prod} + c_{1d}\right\} < \left\{x_{2d} \times E_L(L)_{subst} + c_{2d}\right\} \quad \text{(if vice versa)}$$

with c_{2d} invariably > c_{1d}. For biocatalyst ions, the differences are as such (Table 3.2).

It turns out that there are two groups of elements, namely such which require $E_L(L)$ changes from substrate to product of just more than −0.22 V to overcome enhanced possible binding by formation of additional binding sites (Al, Cu) and another for which this difference must be larger than −0.3 V (Mg, V, Fe(III), Zn, Cd). The "actual" hydrolases belong to the latter class, probably due to the very low $E_L(L)$ values of their most common carboxylate or phosphate products.

Table 3.2 Conditions for "switch" of ligand denticity

Metal ion	Δc	Δx	ΔE_L(L) (V) for closed catalytic loop (minimum value)
Mg	+4.04	12.84	0.31
Al	+12.68	58.22	0.22
V(IV)	+6.8	22.08	0.31
Mn(II)	+2.71	–0.12	(Only feasible for removal of one donor site)
Fe(III)	+13.71	43.81	0.31 (Applies to Fe(III) rather than Fe(II) but haem porphyrins behave somewhat differently (Table 2.3))
Co(II)	4.31	9.23	0.47
Cu(II)	7.34	33.84	0.22
Zn(II)	5.44	17.62	0.31
Cd(II)	3.60	10.95	0.33

Except for this case, catalytic cycles can only be closed if binding decreases either directly or by subsequent protonation of strongly basic ligands (amidate, hydride, alkyl etc.) upon conversion of substrate into product. If the denticity is constant (ethyne reduction to ethylene by nitrogenase, etc.), this avoiding of total product inhibition requires some **change** of ligand sensitivity (plus an appropriate sign off this change), that is,

$$x_{1d}, x_{2d} \neq 0 \tag{3.5}$$

whereas the other case

$$x_{1d}, x_{2d} \approx 0 \tag{3.6}$$

simply translates into product inhibition. Presumably, although the "window of essentiality" appears to be a complete area from $x_{2d} \approx -8 \ldots + 15$, it does contain a "forbidden zone" according to Eq. 3.6 cutting it into two parts. The actual biocatalysts do fulfil the condition by Eq. 3.5, either having considerably lower (Mn^{2+}, Mo(VI)) x_{2d} values than zero or else distinctly > 0 (Co(II), Fe(II), Mg, Zn; $x_{2d} \geq +4$). Among those metal ions having $x_{2d} \approx 0$ (Be^{2+}, Eu^{3+}, Am(III)) the first one even is highly toxic (far more so than e.g. Cd) for both plants and vertebrates, with exactly the unspecific mode of inhibition towards a multitude of enzymes – not just Mg-dependant ones (Kaim and Schwederski 1993; Dulka and Risby 1976) – predicted by the simple model.

Al does not differ from the above central ions of hydrolases with respect to the "critical" E_L(L) value for interconversion of monodentate substrates into bidentate products, hence should be similarly suited for this purpose, and it is known to promote some of the mentioned reactions of carbonyl compounds (hydride transfer in ester formation from aldehydes or reduction of other keto compounds, etc.), and it is very abundant in the environment, including freshwater. So it must be asked why neither Al nor Ti – for which similar arguments can be advanced – are used by any organism. With Ti, probably the extremely low value of x_{2d} hampers formation of a multitude of catalytic cycles in biology. For Al, it is located far above the "window of essentiality", suggesting that the limits of this "window" contain more information on conditions of biological autocatalysis than revealed up to now. The three-functions-role obviously is part of the answer. For monodentate binding the situation is analogous except for $x_{1d} > 0$ being almost non-existent. Beyond inferences from Sabatier's principle and the corresponding "volcano diagrams" (Rothenberg 2008), the general conditions of metal catalysis must be kept in mind: there must be a reaction pathway (spatiotemporal coordinate for translocations of bonds (e.g. electrocyclic reactions), atoms (O in oxidations), or functional groups) which can be followed without overcoming a large energy barrier, with or without influence by a catalyst which thereafter has to be cleaved readily. The formal implications of this general condition were discussed before.

As shown in Table 2.2, electrochemical ligand parameters of biochemical substrates cover quite a range, in fact, the values extend over the entire range for monodentate ligands with OH^-, thiolates up to CO, NO being all relevant. The situation is similar with bidentate ligands, from some –0.30 V for NAs, glycerolaldehyde-2,3-bisphosphate etc. up to some 0.26 V for nicotinamide, free nucleotide bases and other heterocycles such as pyrimidines, and yet higher values for bis-isocyanides. Lever (1990) notes the value of +0.24 V for pyridine and the corresponding (somewhat higher) values for derivatives thereof such as 3-carboxamidopyridine (nicotinamide; +0.29 V) to be measured in water which requires the correction discussed above for CH_3CN solution and applying Eq. 2.4. Notwithstanding linkage isomerism, $(Ru(\mathbf{N}C_5H_4\text{-}C(O)NH_2)_6)^{2+/3+}$ should have an aqueous redox potential of +1.74 V and such in acetonitrile of 0.907 × 1.74 – 0.181 = 1.397 V or (divide the preceding value by 6) E_L(L) ≈ +0.23 V (the carboxamide-bound linkage isomer of nicotinamide

which also can be prepared for various central ions (Balahura and Lewis 1976) would have $E_L(L)$ = +0.03V).

Apart from avoiding product inhibition, reactions must be either exothermic or "pulled" by direct consumption of the product if they should be catalyzed by any means; in addition, binding to the catalytic center must be rather strong to overcome the usually low concentrations of substrates (except for hydrolases, proteases) in biochemistry. There are cases in biochemistry where lots of metabolic energy (ATP) are required for a reaction to take place (irreversibly, not just for activation by means of phosphorylation) even though both conditions apply: reduction of N_2 – bridging in between two Fe centres – to ammonia is exothermic and NH_3/NH_4^+ is used directly after its formation, yet 16 (!) ATP are required to make one NH_3 molecule. As a result, the "window of essentiality" is rather strictly limited to low c values regardless of substrate denticity. The line which holds for divalent essential ions presumably is due to the prominent role of polyphosphates in biochemistry. There is no similar gathering of more highly charged metal ions concerning essentiality.

It is postulated that there is a direct relationship among these phenomena and Eq. 2.4, meaning that the "natural selection of the chemical elements" (Williams and Frausto Da Silva 1996) is mainly due to rules of coordination chemistry, with the selection criteria imposed very early in biological evolution by prevalence of (a) (organic) carbonyl compounds and (b) phosphate-based leaving groups in SN_2-type (Sykes 1977) substitution reactions. If so, the above relationship constitutes the much-searched **causal explanation of the Biological System of Elements**, including the three-functions-rule and Gibbs's phase criterion as limiting conditions for diversities in catalysis and structure, respectively, and quantitative aspects of ligand (substrate) binding controlling the above selection rather than abundance (Al, Ti vs. V, Co, Mo!). The line Eq. 3.2 then represents another criterion for doing catalysis in biochemical conditions. Hopefully, the exact "meanings" of parameters c and x and their obvious yet redox-state-dependent relationship will be revealed by (*ab initio*) quantum chemistry, in addition allowing for calculation of c, x data for other metal ions pertinent to toxicology or radiotoxicology for which then BCF values might be inferred without testing. For now, take $x_{1d} \approx -38$ for the proton as a first hint what data will be like if there is no π binding whatsoever next to some point charge, while the range differences of c between di- and trivalent ions of similar diameters point to the extent of electrostatic contribution, much like in ligand field theory from which the basic redox potential (-difference)s might likewise be inferred.

When there are different possibilities of M-(substrate)^{a,b} binding, there can be different denticities of the substrates and $E_L(L)$ values also, implying that the both substrates will be – if at all – coordinated with unlike strength and mode. When coordination number of the former substrate changes during the reaction, the simple plots used so far will no longer do. So let us first consider a reaction with leaves the denticity as it was before but can be promoted by various metal ions in vitro and in vivo likewise: the stepwise hydrolysis of nitriles or hydrogenation of N_2, nitriles or isocyanides, each of the latter decreasing $E_L(L)$ from highly positive values to +0.08 V at some pair of Fe centers between which the substrate is locked up. Whereas this latter process (hydrogenation of C-N multiple bonds) is catalyzed by Ru species, the reverse reaction (oxidation of primary amine ligands to azomethines and eventually nitriles) can be effected by nickel complexes (Yamazaki and Yamazaki 1990). Stepwise nitrile hydrolysis first affords carboxamides and finally carboxylates; nitrile hydrolases which are broadly distributed in bacteria and plants usually draw upon Co^{2+} (which, unlike in vitamine B_{12}, is not linked to a corrin or porphyrin ring here). There is direct interaction of at least one reaction partner with the metal center; generally speaking, this might either be water, a hydroxide ion or the nitrile/carboxamide itself. There are many Co(III)- (and other, e.g. Rh(III)-, Ru(III)) complexes which promote the same transformation, and thus are considered as bioinorganic model complexes of nitrile hydrolases. Retention of the carboxamide intermediate at cobalt is common, precluding formation of a catalytic cycle at given reaction conditions (carboxamide then is usually bound via N; Balahura and Lewis 1976; Riedel 2004). There, hydroxide or water are transferred to the coordinated nitrile but the reverse reaction would also be conceivable there. In either case, carboxamide or its tautomer or the deprotonated acyl amidate ion will form. In total, excluding carboxylate, there are five possible or actual substrates or intermediates for all of which $E_L(L)$ values are known and thus complex formation constants

– which permit or inhibit formation of some catalytic cycle or secondary hydrolysis towards carboxylate, depending on whether nitriles (or water) can replace the products – can be calculated. Usually, Zn^{2+} or Mg^{2+} would be used in similar hydrolytic reactions; so why Co^{2+} is employed here? Consider Table 3.3.

X will be negative for all biorelevant metal ions if there is monodentate bonding (like in this case), which means that any decrease of $E_L(L)$ during the reaction (or due to ligand deprotonation facilitated by metal complexation) brings about an increase of complex stability according to R-CN < RC(O)**N**H_2 < RC(O)**N**H^- < OH^- (bold letters denote site of binding in the ambidentate (Balahura and Lewis 1976) carboxamide/acyl amidate ligands). Co-dependent nitrile hydrolases thus resemble the more abundant Zn-based hydrolases which convert other substrates insofar as hydroxide or water, rather than the nitrile, are directly bound to the metal ion center, with aqualigands at Co(II) being somewhat less acidic than when bound to Zn^{2+} (Mizerski 1997). If some hydroxoligand reacts with a nitrile – attracted to the catalytic cleft by H-bonding or some polarity gradient – the x_{1d} value of Co^{2+} of – 5.3 combines with the increase of $E_L(L)$ of 0.21 V to a decrease of ligand affinity of $\Delta\log k_{diss} \approx -1.1$, ensuring easy replacement of the acyl amidate product (which then protonates and tautomerizes to yield carboxamide) by another hydroxide ion. With $x_{1d(Zn)} = -8.93$, the effect would be larger than with Co^{2+}, but probably some retention of acyl amidate (water and ligand self-exchange rates in Co^{2+} or Co(III) are smaller than with Zn^{2+}) is useful either to promote the terminal hydrolytic step to afford carboxylate also – rather than releasing the carboxamide directly – or transfer the intermediate to O-donors other than hydroxide (serine- etc. -based alcoholates, etc.). When acyl amidate is released (less fast with Co^{2+} than with Zn^{2+}), it will be a very strong base in either aqueous or lipid (ester-dominated) media, hence both free or coordinated water are rapidly deprotonated which affords the substituting hydroxide ion once again which will then close the catalytic cycle and remove the carboxamide. Esterases rather make use of Zn^{2+} (or sometimes Mg^{2+}), unlike nitrilases, independent of the kingdom of organisms, with carboxylates (and probably also esters[2]) having higher much $E_L(L)$ than OH^-. As the difference in $E_L(L)$ nitrile/acyl amidate vs. hydroxide is larger than ester/carboxylate vs. hydroxide, controlled (rather than diffusion-limited) hydrolytic degradation of the former is better achieved with a metal ion of less negative x_{1d} than Zn^{2+}, namely, Co^{2+}, or Mn^{2+}. In the esterase also direct complexation of ester by Zn would end up with stable binding of carboxylate and thus pronounced product inhibition.

Thus plausible reaction pathways can be distinguished from others which preclude closure of catalytic cycles, with an apparent preference for controlled rather than fastest-possible reactions in metal-promoted biochemical transformations. Strong product inhibition would end up in a stoichiometric rather than catalytic reaction. Going beyond details of reaction mechanisms for single reactions (e.g. Gray 1975; Lipscomb 1982; Riedel 2004), more general statements can be derived from the c/x maps for either denticity of substrate/product ligands: there is a broad range of x_{2d}– or x_{3d} values for the set of essential biocatalytic elements, with x having either sign while x_{1d} is always negative. Now, Fig. 3.1 shows that the window of essentiality than is placed around $x_{2d} \approx -8 \ldots +15$, while for the monodentate case (Fig. 3.2) it is in the upper right edge of the diagram, that is, at maximum c and with x_{1d} being not too much lower than zero (about −10 to −4). Also notwithstanding the fact

Table 3.3 Electrochemical ligand parameters of the five species which may be involved in nitrile hydrolysis (substrates, products, intermediates). Whereas nitriles are not tightly bound, hydroxide binds to either metal center more strongly than both carboxamide and the corresponding base, acylamidate. Here, however, relative Brønsted acidities of water and carboxamides must be taken into account. Water can only be transferred to a nitrile before being deprotonated at Zn(II) but not at Co(II) which means that this is not the actual mechanism of biochemical nitrile hydrolysis. Negative values of $-\log k_{diss}$ are given in brackets because they correspond to unstable complexes which hardly persist in water

Ligand	Electrochemical ligand parameter $E_L(L)$ (V)	$-\log k_{dissCo(II)}$	$-\log k_{dissZn}$
Nitrile	ca. +0.36	(−0.7)	(−3.5)
Carboxamide RC(O)**N**H_2	0.03	1.0	(−0.56)
RC(O)**N**H^-	−0.38[a]	3.2	3.1
H_2O	0.04	0 per defin.	0 per defin.
OH^-	−0.59	4.3	5.0

[a]Calculated value using electrochemical values by Chou et al. (1994)

[2] For esters $E_L(L)_{R'\text{-}COOR}$ can be estimated from charge change effects due to (de-)protonation and H/alkyl similarity for uncoordinated substituents (cp. amines):
$E_L(L)_{R'\text{-}COOR} \approx E_L(L)_{R'\text{-}COOH} \approx E_L(L)_{R'\text{-}COO^-} + 0.5$ V, that is, about +0.3 V (somewhat less than for nitriles).

that only some 30–40% of enzymes work by direct metal ion catalysis, this supports the assumption that ways of change of substrates in most general metabolic pathways control the possible sets of catalytically active elements. Apart from those elements like V which are essential in heterotrophic organisms (animals, fungi) mainly oxidizing external organic matter, or in certain, strongly reducing environments (W), the situation is balanced in the way that either direction of redox transformation, entailing changes of $E_L(L)$ in either case, must be effected "upward" or "downward" by often the same metal biocatalysts. Also taking account of microscopic reversibility in catalysis and the fact that some enzymes can be employed "the other way round", e.g. formate oxidase for achieving CO_2 reduction at electrodes, this requires further scrutiny which will be the topic of the next chapter.

There is an old tradition in quantifying terms of coordination chemistry in order to predict complex formation constants from sets of empirical data: Martell et al. (1985) give a review of (now even more) historic attempts to estimate binding stabilities for mostly monodentate ligands, introducing a ligand parameter a (see Section 2.2.3). Then (e.g. Larsson 1934; Bjerrum 1950; Irving and Rossotti 1956) complex formation constants were usually linked to other kinds of chemical equilibria which involve (some of) the same species, e.g. to acidities of the acids corresponding to the protonated ligands (say, NH_4 or Me_3PH cations, HF, HCOOH or Ph-SH), linking metal complexation properties to acidities of the corresponding acids H-L or HL^+ of anion or neutral {e.g. NH_3, $P(CH_3)_3$} ligands. H^+ of course also is an electron acceptor for which c and x parameters can be calculated for both neutral and anionic ligands (corresponding bases), cp. Table 2.3 and Fig. 3.2. Irving and Rossotti (1956) also made use of (aqueous) acidity data for predictions of metal complex stabilities but defined another term *b* and did correct for solvation effects in mixed solvents.

Now having several empirical parameters in a "fitting" equation, it is no longer possible to calculate some term *a* directly from general or averaged data; apart from this, this would be beyond and besides the ends of this work – our task is to construct a parameter set which describes the biochemical behaviour of metal ions while prediction of complex formation constants can be also applied for estimating thermodynamics of metal ion transport and retrieval in a terrestrial ecosystem.

For linking metal complexation properties to acidities of the corresponding acids H-L or HL^+ of anion or neutral {e.g. NH_3, $P(CH_3)_3$} ligands in very much the above manner, and with c_{1d} and x_{1d} also calculated for H^+, we can compare those older data even though these usually were restricted to quite few ligands and metal ions; for example, Bjerrum considered CN^-, NH_3, pyridine and some primary to tertiary amine ligands. Bjerrum postulated the following equation chain (3.7)

$$\text{Log } k_{ML} / \log k_{HL} = a = \textit{constant} \tag{3.7}$$

which permits to calculate a values for any of the above ligands by using the respective values $c_{1d} = -6.52$ and $x_{1d} = -38.05$ for the proton. Then,

$$\begin{aligned} &\left\{c_{1d(M)} + x_{1d(M)}\left[E_L(L)\right]\right\} / \\ &\left[-38.05\, E_L(L) - 6.52\right] = \\ &- \left\{x_{1d(M)}\left[E_L(L)\right] + c_{1d(M)}\right\} \\ &/\left[38.05\, E_L(L) + 6.52\right] = \textit{constant} \end{aligned} \tag{3.8}$$

and thus

$$\begin{aligned} &- a \times \left\{c_{1d(M)} + x_{1d(M)}\left[E_L(L)\right]\right\} \\ &= 38.05\, E_L(L) + 6.52 \end{aligned} \tag{3.9}$$

which is equivalent to

$$\begin{aligned} a = &\left[-38.05\, E_L(L) - 6.52\right] / \\ &\left\{c_{1d(M)} + x_{1d(M)}\left[E_L(L)\right]\right\} \end{aligned} \tag{3.10}$$

for the above ligands cyanide, ammonia, some amines and pyridine. Equation 3.10) affords quotient a which Bjerrum postulated to be constant and typical of a certain ligand. For $E_L(L) \approx 0$ (e.g. CN^-, NO_2^-, oximes, porphyrine^{2-}), Eq. 3.10 simplifies to

$$a \approx -6.52 / c_{1d(M)} \tag{3.11}$$

the most familiar 3d transition (dicat-)ions having $x_{1d(M)} \approx -8$ (Table 2.3) which gives the following values:

pyridine	–2.1
NH_3	+1
CN^-	–5

Of course, this approach may be extended to multidentate ligands such as amino acids, dicarboxylic acids

or chelating amines; for bidentate ones with biorelevant metal ions, c and x are coupled to each other, as noted above, from Eq. 3.2)

$$x = 3.545 \times (c - 3.085) \tag{3.2}$$

and thus, from Eq. 3.10, for bidentate ligands attached to divalent cations:

$$a = \left[-38.05\, E_L(L) - 6.52\right] / \left\{c_{2d(M)} + \left[3.545 * \left(c_{2d(M)} - 3.085\right)\right]\left[E_L(L)\right]\right\} \tag{3.12}$$

with the second deprotonation steps of the chelating ligands (pK_{a2} = 4.4 for oxalate, 9.7 for glycinate etc.) and c ≈ 5, x ≈ + 8 for "average" biocatalytic metal ions:

oxalate	0
malate	–0.25
glycinate (prolinate)	–0.85
hydroxamate	+0.17

There is a biochemical and biogeochemical message in this use of Bjerrum's ligand parameter a which is the actual reason of this seemingly exotic comparative excursion.

There are equilibria between protonated and metallated forms of various ligands, e.g. in the rhizosphere or during digestion in vertebrate guts, and during chemical evolution the change of ligand kinds and complexities – even allowing for polymer backbones – influences metal distributions between aqueous media – not only acidic ones – and solid going-to-be biomasses (see Chapter 4). The chelator ligands used by both green plants and fungi have Bjerrum a values around **zero**. For a = 0 (oxalate), metal affinity (and thus the chance/efficiency of metal ion retrieval by roots, mycelia and the like) would not at all depend on the acidity of the corresponding acid. This makes sense: by delivering such ligands to the soil (liquid), plants, fungi or soil bacteria have the best chances to obtain the required metal ions independent of soil (water) pH. As adaptation to extreme environments like peat bogs is limited, yet (which is shown by rather low biodiversity there), this "trick" does not make plants independent of the set of c; x values in the window of essentiality. It is straightforward to repeat the above calculation for the four relevant ligands and c; x of Mg, Mn, Zn (including some offset from the line expected from Eq. 3.2 vs. those of metals not needed by green plants but by animals (V, Co) or only by very special plants (Ba, Cd) to test for this possible selectivity under acid stress which obviously is pertinent to consequences of soil acidification. For this purpose a* values are defined which refer to uptake chemistry of a single one of these cations (Table 3.4).

It is interesting that the above function for competition among metal ions and protons for some ligand yields very similar results for metals on either side of the window of essentiality such as Cd and Cu^{2+}, with the divisor being 3.57 and 3.59 in these cases. Oxalate gives an almost perfect compromise which can serve the metal demands of both plants (including green algae) and fungi; probably this is why it is also used by lichens, yet not capable to protect them completely against acid (SO_2) impact. Glycinate, prolinate and similar amino acids produced by grasses give a larger selectivity in M/proton competition particularly for "photosynthetic" metals Mg and Mn. Perhaps this trend to use ligands which do not discriminate too much against protons would control the functions open to metal ions taken up by plant biomass at least from the moment plants took root on solid land (Silurian).

As a rule, **relative** stabilities among members of certain series of complexes, say halogenoargentates(I) (X = F to I) or hexacyanometallates(III) (M = Ti, Cr – Co,

Table 3.4 Bjerrum's a parameter as calculated from c and x and corresponding properties of the proton. The ligands most often given away by both green plant roots and fungal mycelia produce a ≈ 0, making uptake of metal ions less sensitive towards pH changes even though certain of these ligands are fairly readily protonated, i.e. are considerable Brønsted bases. This does not hold for those amino acids which are employed by grasses, mosses mainly, pointing to decreased selectivity

Ligand	$a_{average}$	a_{Mg}	a_{Mn}	a_{Zn}	a_{V}	a_{Co}	a_{Ba}
Oxalate	0	–0.015	–0.02	–0.01	–0.01	–0.01	–0.02
Malate	–0.25	–0.46	–0.53	–0.40	–0.36	–0.39	–0.77
Glycinate	–0.85	–1.35	–1.56	–1.18	–1.05	–1.14	–2.27
Hydroxamate	0.17	0.32	0.37	0.28	0.25	0.27	0.54

vanadium(III) rather forming $(V(CN)_7)^{4-}$ (Cotton and Wilkinson 1981)), do not vary upon change of solvent (Golub and Köhler 1979) even if changing into aprotic or fairly non-polar solvents although absolute stabilities may alter so much as to make some of them unobtainable from strong donor solvents like water, formamides or acetonitrile. Thus rather non-aqueous compartments of biosystems, like lipid depots or chitinous shrouds will interact with metals in biology in a way which is not too different from the behaviour of aqueous interiors of cells or fluid conduction systems (phloem, xylem, arteries, veins, vacuoles etc.)

3.3 Phase Structures and Histology Revisited

Living beings, particular metazoan ones, are highly complicated systems, sporting a large number of different molecules and often also mineral phases (deposits, internal or external support structures). Their organs and organelles differ from each other insofar as they are constructed from

- Different cell structures
- Have different chemical compositions, for example, it can be anticipated that photosynthetic organs of plants bear higher concentrations of both Mg (chlorophyll, *rubisco* protein which does attach CO_2 molecules to ribulose-bis-phosphate) and Mn (photosystem II). The above photosynthetic organs are connected to
- Liquid volumes in- and outside the plant organisms, including ambient or soil waters, liquor in vasculae or – for succulent plants – in storage tissues
- However, this same criterion of autocatalysis also can – and effectively does – limit internal chemical complexity even though the latter might appear to be very high in living beings. This particularly holds if histological differentiation is very pronounced. The corresponding conditions for internal coexistence of chemical elements and biochemical cycles extend somewhat beyond the discussion of hypercycle (Eigen 1971) stability given by Clarke (1980) in terms of stoichiometric network analysis (SNA). Histological diversity of multicellular organisms, is linked to chemical heterogeneity and implies that statements from SNA and estimates of complex formation constants cannot be taken as "stand-alones" for a biochemical understanding of entire metazoans since the latter are not chemically homogeneous – or constant in time. For example, both seedlings and trees (Sterner and Elser 2002) or tadpoles and frogs differ in composition even in the "classical" C/N/P ratios level of ecological stoichiometry, with the latter amphibia depending on a "switch" in iodine (thyroxine formation) and Cu biochemistries (promoting oxidation and modification of phenoles and phenyl alanine) even to undergo metamorphosis. Moreover, tadpoles and frogs differ in their diets considerably (insects vs. algae, detritus). As thus there must be some chain of events, induced by hormons, which change I and Cu uses (and probably others, also) in the amphibium, there is neither temporal nor spatial (thyroid gland) continuity in element use.
- Taking spatial heterogeneity among the organs into account implies there are a kind of different **phases** – in the physicochemical rather than the temporal meaning of this term – which act differently although they do – and must for survival of the metazoan organism – cooperate. This reasoning provides an additional formalism or approach to describe (and quantify) histological and functional differentiation within a multicellular organism, including a vascular plant. Once e.g. different organs of a plant, fungus or animal are taken to represent different physicochemical phases, the phase rule by Gibbs can be applied to the stability of living beings and their single organs under given biochemical and geochemical constraints: only if there are still degrees of freedom – which in turn limit complexity – the organs can fully coexist and even cooperate in a way which allows for survival and reproduction of the entire metazoic organism. As this approach also is novel to the realm of biology, it shall be discussed first among the methods.

Obviously metazoans like higher plants discussed are capable to sustain life if and only if almost all their organs can exist for long periods of time while promoting their corresponding biochemical activities close to each other at an identical temperature. Although internal gradients may be sustained, facilitating this condition, active transport of ions or even homoiothermy imply additional use of metabolic energy. Some components lacking "primary" functions may increase the number of degrees of freedom or that of possibly

co-existing phases, hence proper functioning of so complex an organism depends on the presence of these elements, too. So these become essential in a very special way and the classical concept of essentiality can no longer be maintained in euryoicuous species – which demand for a large number of degrees of freedom to respond/adapt to changing ecochemical and other conditions. The very differences among organs and tissues by which these may be distinguished in histological or biochemical experiments to have them considered as different physicochemical phases help to meet the conditions for essentiality, too. It is obvious that in different organs or tissues the same compound (substrate) may be used in different ways, and there also should be unlike catalytic transformations and agents each influenced by differing intracell and intraorgan parameters such as pH, redox potential, mixture of ligands and the extent of photochemical effects (large in leaves or needles, essentially zero in roots), all of them influencing both redox speciation and the capability to adapt to different and spatiotemporally varying ecological and ecochemical conditions. For example, in succulent plants there is a diurnal cycle of increasing and decreasing acidity caused by photochemical production (via the catabolic Krebs cycle) of malate/malic acid (Marschner 1986; Heß 1999).

Additional essential elements – which distinguish all groups of metazoans from the protozoans including the eukaryotic ones, like yeasts or protists – provide chances

(a) To fulfill the principal biological functions by a lower number of different tissues by increasing the chemical complexity and number of different transformations within one tissue, say an animal liver, with a correspondingly reduced number of phases, while they in addition
(b) Help to produce yet other substances or sequester them from the environment and thus increase component numbers

Either way the ecological potential of this species is increased with the number of degrees of freedom: the plant or other organism may better adapt to changes or different static conditions. To give an extreme example with animals, **sponges** possess just about three different kinds of tissues, one producing the skeleton, another for nutrition (inner side) and the third for reproduction. Among sponges living in the same regions, there are large differences in essential element patterns, including large amounts of rather uncommon bioelements (Si, Sr, not just in the skeleton); possibly some of them are also involved in syntheses of "exotic" biochemical products frequently found in sponges but not other animals (e.g., halophenols, polycyclic terpene or perhydropyrene isocyanides, involving isothiocyanates and cyanides as precursors and interconverting with the corresponding multiring isothiocyanates by both sulfur atom abstraction/addition and cleavage of the multiring hydrocarbon/pseudohalide bonds (Simpson and Garson 2004) but avoiding formate esters as their "logical" precursors). So sponges should be able to adapt to very different conditions which they do indeed, displaying considerable chemical variations, perhaps related to the above and other exotic ligands in their biomasses. The high level of histological differentiation in vertebrates (more than 100 different kinds of issues in an organism) thus prompts an answer to questions or propositions like that put forward by Horovitz (1988); "Is the major part of the periodic system really inessential for life?", Nielsen or Anke. For reasons of both chemical versatility and abundance, **not much more than 35 elements** may be essential in terms of catalytic activities, yet others may exert some stabilizing effect in the above sense. The conditions for such elements to contribute to fulfil Gibbs's phase rule by increasing the number of components yet must be more precisely investigated.

It is both possible and commonplace in anatomy and histology to **count** the different organs or tissues which exist in some species. Now, these different organs differ – and can be distinguished – not only in terms of histology or cell anatomy: each of them in addition forms some (usually solid) **phase** which (*vide supra*) has to coexist with the other phases in the same organisms in a stable manner. In terms of physical chemistry, such a phase is homogeneous, whether it be a solution or a solid phase. It may even be a mineral phase produced by a plant (SiO_2, $CaCO_3$, $SrSO_4$ (some diatomees) or whewellite CaC_2O_4) but it can also (more frequently) be an organ: tissues may be grown from isolated cells because these are almost identical among each other, identical also in terms of chemical composition but different from other organs. This is very similar to inorganic multi-phase systems: e.g. by proper selection of salt concentration and temperature, aqueous rock salt (NaCl) can be made to form four coexisting phases out of just two compounds, NaCl and water. These four phases are vapour (practically

neat H_2O), pure water ice, likewise solid NaCl (or NaCl hexahydrate below 273.3 K) separating at the bottoms and, of course, liquid aqueous NaCl solution in direct contact with all the others, steadily exchanging matter on a microscale.

For three compounds, two of them being liquids which are only partly miscible, and the third a solute soluble in either, e.g. water, cyclohexanone and sodium thiocyanate NaSCN, even five phases may form in appropriate conditions:

- Vapour, containing water and some cyclohexanone as a mixture
- Cyclohexanone-saturated water with dissolved NaSCN
- Water-saturated cyclohexanone with dissolved NaSCN, the solutes keeping it liquid although it is in contact with
- Neat solid cyclohexanone and eventually
- Undissolved, likewise solid NaSCN

The phase rule (eq. 3.1) implies there cannot be water ice in addition, but would replace one other (or, given the rules, in fact, two other) phase(s) (namely there would then, at lower temperatures, exist neither liquid water-saturated cyclohexanone with dissolved NaSCN nor solid NaSCN)

The key statements are that the systems are dynamic, in constant exchange of materials (provable, e.g. by isotopic labelling) even though there may be no net transfer of material among the phases (and cannot be in cases of zero degrees of freedom; also "freezing" the relative shares of the phases) and moreover may consist of some (generally speaking, arbitrarily large) number of compounds each, notwithstanding e.g. ionic dissolution like with the NaCl and NaSCN examples. Yet, the maximum number of phases depends on that of contained components (chemical compounds), the vapour forming one phase only as all gases are miscible (but might react chemically to produce solid or liquid by-products and thus additional phases: say a hot mixture of water vapor, H_2S and some SO_2 will separate sulfur droplets as some second phase, two out of up to six). Please note that, although in the former example the number of involved chemical elements (four: H, O, Na and Cl) corresponds to that of maximum phases, this does not hold for the second one: here there are five phases at most, but containing six elements: C, H, O, N, S, and Na. Likewise two-or three-component systems with less elements (e.g. $Ba(OH)_2$/water or water/3-pentanone/oxalic acid (C, H, O)) would afford the same number of phases given by Gibbs's rule.

All these phases are chemically interconnected and their (large yet finite) chemical complexity limits the possible number of phases (higher animals contain some 200 different kinds of tissues); every distinct organ can be taken as a single phase, as can be all the liquids separated by membranes inside an organism and – for plants – two different gas phases (air around the above-ground parts of the plant, soil gas around the roots (usually still containing oxygen except when the soil is very wet, but enriched with CO_2, N_2O and sometimes other gases with respect to ambient air)) which are linked by the very plant (metabolism and matter transport processes) and thus actively coexist. Every such phase in itself is composed of one or (more often) several to many components, that is, chemical components independent of each other (Wedler 1982). "Independent" here means that the corresponding chemical species can form a phase of its own or dissolve/partition in(to) some liquid or solid other phase without taking additional other chemical species with it. For example, NaCl can exist as a distinct phase (solid or molten rock salt) or dissolve into water, acetonitrile but chloride ion cannot do so for itself because macroscopic phases must remain close to electric neutrality, thus chloride will co-transport the same amount of Na^+ ions upon dissolution or partition in one or among several liquid phases: NaCl is independent while chloride is not, and the same holds for all other kinds of ions and for compounds which exist only in small amounts given the presence of some excess of the species they form of (e.g. nitrous acid HO-NO/HNO_2). Bioorganic materials are unstable towards an oxidizing atmosphere and often towards hydrolysis but persist for reasonable periods of time thus can be treated as forming distinct physicochemical phases.

Such biochemical peculiarities include those of secondary metabolites such as alkaloids; the composition of alkaloids makes them efficient – often chelating ligands. Although concentrations of such alkaloids usually are small (e.g. 1.5–6% of caffeine in coffee beans), there is a considerable (both mass and stoichiometric) excess with respect to metal ions. Accordingly, alkaloids can be expected to influence both metal partitioning and $E_L(L)_{eff}$ (because values for N-heterocycles are rather high) of leaves, fruits etc. considerably. Indeed, for *Taraxacum officinale* (dandelion) leaves,

where the alkaloids are located in the shoot mainly, a very negative $E_L(L)_{eff}$ of –0.27 V is calculated, possibly in (formal) "compensation" of unbalanced, alkaloid-biased metal transport in the milk sap in the shoot.

It suffices to know all the parameters (pressure, temperature and amounts of the components) but one to have a multiphase system fully defined (Sillen 1967), and this likewise holds for al the organs, the solution phases (cell sap, blood or xylem liquid, etc.) and one or two gas phases referring to an organism. Since and as long as all the phases coexist, the number of degrees of freedom F in a multiphase system consisting of K components and P phases will be

$$F = K - P + 2 \tag{3.1}$$

(Wedler 1982)

Upon some change of ambient conditions in a multiphasic chemical or physical system, certain phases may vanish (cp. the three-component water-cyclohexanone-NaSCN system if cooled so much as to make neat water ice appear) or convert into other ones. If F would become negative ($F < 0$) otherwise, like in this example, some phases even **must** disappear due to condition (3.1). This disappearence of phases might then mean collapse of gas (vapour) phases, dissolution of a distinct salt-based bottom solid or the like. Regardless of chemical complexity (which is quite remarkable in living beings), the number of coexisting phases cannot get arbitrarily large (Sillen 1967).

As shown before, Gibbs's phase rule poses an upper limit to the number of such phases which are homogeneous in itself (say, histologically homogeneous parts of tissues or organells inside cells) but differ from outside species concerning chemical composition or (liquid, solid, gaseous) state. It is a precondition for this approach rather than an obstacle that organs are in constant exchange of matter. The phase rule does apply only if exchange takes place: systems which are not connected by matter flow cannot attend equilibrium and thus are no coexisting phases, hence will not either limit their numbers. As for disappearance of some of the phases, this is acceptable in living beings only if can be integrated into lifecycles (removal of leaves, degradation of reproductive organs in seasons other than those of growth and reproduction); otherwise the biological system will show grave damages (death, heavy damages).

The task remains to count corresponding phases (organs/kinds of tissues) and chemical components in (those) higher plants we deal with here; as there are histological and biochemical differences among plant species, the pertinent data will hold for few species or taxa at most. Autotrophic – both photoautotrophic and chemoautotrophic – organisms are capable of a multitude of "sophisticated" chemical transformations; accordingly, the number of essential components (essential in terms of both classical biochemical definitions and SNA definitions) (Clarke 1975; Eiswirth et al. 1991b) thus should be similar or even smaller (essential compounds generally consisting of several elements) than that of the essential elements (≥16 in higher plants), say 15–20. Using Eq. 3.1)

$$F=K-P+2$$

once again, the number of remaining degrees of freedom can be determined. Corresponding degrees of freedom include temperature, pressure (in aquatic organisms) or of environmental concentrations (and thus, amounts of supply) of both essential and non-essential elements. These parameters combine with the number of components to limit the number of phases which is "acceptable" in higher life-forms; heterotrophic organisms can be more complicated histologically exactly because the number of required components is higher as these are not able to synthesize the plethora of bioorganic materials from simple oxospecies. Besides of chemical complexity in either "internal" biochemical transformations and required forms of supply, the "necessary" number of remaining degrees of freedom depends on the capacity of an organism to adapt to varying conditions (euryoicy being the capability to survive and reproduce in different climatic and chemical circumstances), and in addition accumulation of elements which are not involved in or required for promotion of biochemical reactions but which occur in plant biomass in substantial amounts will likewise stabilize the system by increasing the number of components K. Further pursuing this line of argument, the number of remaining degrees of freedom is crucial for euryoicy and, more generally speaking, determines the ecological potential of some species: the more simple the organism is in histological terms (the minimum being three to four kinds of cells in sponges) and the larger the number of essential species on the other hand, the larger also the number of remaining degrees of freedom will be. Regardless of their histological simplicity, even sponges which coexist

in some habitat, e.g. close to the shorelines of the Gulf of Bengal, may strongly differ in essentiality patterns plus other enriched elements (Sr, Ti, …), the degrees of freedom probably being "invested" into components required for synthesis of uncommon metabolites such as haloorganics and isocyanides (which also may sequester trace metals from seawater); the same holds for bryozoans which also form haloorganics (Gribble 1992), presumably using haloperoxidases or some variety of Sandmeyer's reaction (that is, Cu).

By this approach essentiality of metal ions can be defined in a semiempirical way: ranges of parameters c and x can be identified which are "suitable" for biochemical, biocatalytic essentiality even without knowing biochemical details of function or mode of catalytic activity. These statements then either hold for essentiality concerning all organisms or others just required by those living in uncommon conditions of redox potential or temperature, say (essentiality of) W in clostridia or archaea. Given sufficiently many complex formation or equivalent association constants are known for some metal ion or other electrophile, the two parameters c and x can be determined and give an idea concerning properties of that electrophile (Fig. 2.1). Beyond estimating stabilities of the complexes, the distribution of c and x in a parameter space plotted vs. essentiality or related biochemical features provides criteria which relate to specific suitability for promoting biocatalytic transformations of quite different substrates – or block it once taken up by some apoprotein (generally toxic elements).

Nevertheless, it should be pointed out that describing organs of plants or animals as physicochemical phases is a kind of model: even though chemical differences may cause different transformations by very similar biomolecules, these "phases" do not differ with respect to their aggregate states. Given the compositions of metal resources in soil, fresh- or ocean waters and coordinative selectivities, the question arises whether there could be some "minimal" organism which produces the abundance patterns Fe ≈ Zn > Zr > Cu > Cd > Mn > Cr > Co > V, typical for animals (Kaim and Schwederski 1993), or Ca > Mn > Fe ≈ Al > Ba > Cu in many kinds of plant leaves/needles just by using a single kind of transporter and its metal selectivity due to unlike complex stabilities. Such an organism would provide an ideal accumulation bioindicator. While "harmless" non-essential elements, including REEs (Fränzle 2008), do actually fractionate like this in biomass, allowing for attribution of an effective electrochemical ligand parameter to biomass samples (see above), some selective transport – limiting inflows of Mg from seawater (at 53 mM/L (Nozaki 1997) too much even for photosynthetic organisms) or of Cu (producing very stable complexes) from any environmental compartment – is required to keep an organism working and healthy. In addition, Fig. 3.2 suggests **maxima** of BCF values to occur as a result of soil or (organic ligand-derived) aquatic chemistry. Except for Mn accumulation in marine bacteria, the highest BCF values (several times 10^5) were observed for a multitude of polyvalent metal ions including Fe, Al, V, REEs and U in iron bacteria (*Leptothrix ochracea*) from a Czech freshwater spring (Fränzle and Noack 2008, unpublished yet, and Fig. 2.12). This organism thus closely approximates an ideal bioindicator for trace metals in water, with an $E_L(L)_{eff} = -0.16$ V that is close to that of photosynthetic organs of several trees and grasses.

3.4 Scope of the Essentiality Model

Extending the scope of view to the ecological position or niche of some species, for estimating numbers of degrees of freedom or thermochemical variability counting organs or distinguishable tissues (+ biofluids, mineral phases like magnetite, silica) in a plant or its seedling form will not do (likewise in animal (-larvae)). If related to the potential to spread, strategies of reproduction (including a possible switch between sexual and asexual reproduction) also need to be considered: seeds and root nodules are heterotrophic in the beginnings, the number of co-existing phases being lower also as far as no photosynthetic organs have developed. It is intriguing that a considerable number of oxophilic yet not essential elements (e.g., REEs) are strongly depleted in seeds such as grain (Emsley 2001; Kabata-Pendias and Pendias 1984). Generally speaking, the degrees of freedom correspond to both thermal and ecochemical (soil element patterns, salinity and salt composition for aquatic plants) adaptation ranges of one species.

It appears straightforward to use electrochemical data (redox potentials) for proteins containing Fe or Cu vice versa to estimate the strengths of complex binding of

apoprotein and substrate binding in redox-active metalloproteins. Eventually returning to SNA arguments, "typical" biochemical cycles were attributed to type (reaction network topology) 1B according to the responses of human cells or isolated DNA towards cytostatic agents (Fränzle and Markert 2003). The 1B type (Eiswirth et al. 1991b) denotes a critical cycle which is capable to induce and undergo chemical oscillations even if there are no throughflows of reagents through the reactor (B = *batch*, meaning a reaction in a closed beaker). While living beings are generally throughflow reactors, there are few oscillations in biochemistry which yet is no contradiction to the above identification as oscillation need not necessarily occur in a batch (or throughflow) system if any one of the conditions are not met: pH, other concentrations, temperature (most – also 1B kind – oscillators operate in highly acidic conditions (Pota and Stedman 1994)). The polyfunctionality of many biological autocatalysts might bring about very high autocatalytic orders and thus strong AC cycles, unlike that of the oxonium ion H_3O^+ which uses to be the only autocatalytic species in man-made oscillator or chemical wavefront systems, except for "nested" AC structures such as Eiswirth's type 1CS. In addition, the above polyfunctionality in biochemistry often goes along with homoeostatic organization of resorption, concentration and distribution (Cu, Fe) or that of elements which are distinguished by an extreme reciprocal coupling among the AC cycles (non-metals like C, H, N and S). Although this basic kind of feedback loop was deduced from cell budding in humans only, it is safe to state that this very kind of autocatalysis likewise holds for green plants and other organisms: any modification of this feedback pattern – at least after assembly of first metazoans which took place well before separation of the last common ancestors of all plants, fungi, animals and protists – would have been a lethal one as it would have *reversed* the effect – and thus the "meaning" – of chemical signals linked to resource feedback in autocatalysis. Thus any change of this pattern and of regulation modes on this most fundamental level would cause both genetic and geochemical (exogeneous) signals to induce paradoxical responses and thus cause metabolism to break down completely. As 1B thus was settled before plants and animals separated in evolution, it must also hold for autocatalytic feedback in contemporary green plants (recall that AC feedback with many elements essential to green plants cannot be traced "downward" to the level of molecules transporting or processing (redox enzymes, ligases etc) this very element). To give a summary of the model to "explain" essentiality patterns which was advanced in this work, recall the following points:

1. Stability and multitude/diversity of metal-ligand bonds which can be formed by some metal ion in redox-neutral to oxidizing aqueous media and its biocatalytic "feasibility" and thus possible essentiality are linked to each other.
2. The above multitude/diversity of metal-ligand bonds and of (bio-)catalytic transformations is given by terms (parameters) which are typical for the given metal ion (although c and x values change with oxidation states (Fe, Ce, V, etc.) and c;x are linked by correlations for given oxidation states).
3. For biological reproduction the construction of autocatalytic cycles is required which reduces the options to use broadly diverse metals (elements in general) in biological systems.
4. Tissue diversity (histological complexity) acts likewise.
5. Producers (green plants, chemolithoautotrophs) make use of less many different chemical elements than consumers even though their metabolic complexity is larger; perhaps histological and biochemical complexity in plants is even limited by grassing.

It goes without saying that both the model and the above (hypo-)theses require testing and critical scrutiny. For the stunning distributions summarized in Figs. 3.1 and 3.2, there are theoretical arguments backing them in quantitative coordination chemistry. Because there are data for quite many non-essential elements as a kind of control, it is highly likely that regressions like Eq. 3.2 and the "windows of essentiality" actually correspond to necessary conditions of biological catalysis using metal ions (inside some protein matrix). This general relationship between biological use of metals and complex formation involving them is due to

(a) Certain metal ions being required by all organisms on Earth.
(b) Accumulation and transport of metal ions in organisms taking place by means of coordination chemistry as both main and minor components of organic biomass bear (also) ligand properties.
(c) Eventually catalysis by enzymes is accomplished to a substantial part by metal complexes as intermediates, with regulation of enzyme activity involving metal (often Ca^{2+}) complex formation like the induction of certain peptides/proteins also in many cases where the very protein function is independent of any metal ion.

Regardless of chemical details (or chemistry being involved at all) or of the topological complexity of the reaction networks, the theorems of stoichiometric network analysis must fully apply to biology/biochemistry as we deal with autocatalytic loops. There is a rather limited (some 600 mio. years, with fossil evidence commencing in upper Ediacarian formations) history of histological differentiation, yet it is coupled to an increase in numbers of essential elements with respect to bacteria, protists and other unicellular organisms (Table 2.1). Though the three-functions-rule points out the problems in introducing some novel element to biology, it is most readily achieved by simple addition – via endosymbiosis or similar ways of association.

The different substructures now linked to each other then will engage in exchange of matter and organize coupled chemical reactions, thus becoming phase assemblies to which modes of behavior and stability criteria derived from Gibbs's phase rule will apply. SNA and Gibbs's phase rule jointly reveal the obstacles produced by integration of hitherto autonomous (and autonomously reproducing) cells into some hierarchically cooperating structure. Although they also can reproduce only within this hierarchy from now on and different patterns of essential elements add up, taking the three-functions-rule for granted in the metastructure now (e.g., Mn in metazoans), obstacles now come from the existence of different phases (histologically different organs or cell assemblies). The process of association via e.g. endosymbiosis alters both fluxes and required amounts of elements and – via novel modes and ends of internal coupling – autocatalytic orders of elements and other indispensible components (essent. cofactors, "vitamins") whereas, in this very moment, structural complexity does increase by combining parts of biomass which differ with respect to all biochemistry, morphology and histology. The former factor does optimize use of matter, while there apparently remains some limitation of essential element patterns/numbers in consumers subjected to grassing. After producer (either photosynthetic or chemoautotrophic) integration, this does not hold any longer: all zooxanthelles in corals, lichen symbiosis, H_2S-oxidizing bacteria which thrive as endosymbionts in tube worms (*Riftia pachyptila* and others) and mollusks (*Calyptogena*) at "black-smoker" sites, obviously bring about Sr and V essentialities, respectively, in the former two cases which "plain" green plants cannot do. The advantages of symbiosis and endosymbiosis are, however, partially outweighed by possible dynamic instability within the cooperation structure and – by loss of degrees of freedom means decreased chances to balance variations in physicochemical environmental parameters.

Two simplifying assumptions were done for the sake of complexity reduction. In a metalloprotein there exist other coligands besides water, namely amino acid side-chains (often three imidazoles from histidine or carboxylate (Asp, Glu) or phenolate (Tyr)) and cofactors like porphyrines ($E_L(L) = 0$ V; Lever 1990), but never so diverse as to bring about M-centered chirality, plus pterines, flavines, metal sulfide clusters ($Fe_3M'S_4$ and others) etc. Except for data on c and x values of iron(III)porphyrine species (in fact, haem proteins), some CoL_5 species and the last (sixth) coordination site of some essential metals, 2nd-order effects due to coligands are not yet addressed on this modeling level: they were not dealt with either in other attempts to link (biocatalytic) essentiality to e.g. (intermediate) absolute hardness of the corresponding metal ion electrophiles (Williams and Frausto Da Silva 1996); here even "naked" cations were considered rather than aquated ones. Nevertheless, some "window of essentiality" here also emerges, with the "borderline" (neither hard nor soft) metal ions. Essentially, the above authors treated the fixation of a substrate to the center of some metalloprotein like that to an homoleptic aquaion of the same element and oxidation state while in biological systems 2–3 (4 in haem systems, Ni porphyrines or Co corrines) coordination sites are relatively strongly fixed to species (donor sites) other than water.

We discussed the implications and conditions (in terms of $E_L(L)$) for labile equilibria between aquation and higher apoprotein complexation of some metal ion inside the protein and its ramifications for catalytic activation before. The other neglected feature are solvent effects brought about by some highly unpolar immediate surrounding of the metal ion, e.g. nearby Phe, Leu, Ile or Val residues or embedding of the active site/cleft into a lipid membrane, which spells a very low local chemical activity of water. This will alter equilibrium constants of complex hydrolysis, used to calculate c and x values, but, given the series of complex formation constants being almost identical in different solvents, larger effects may be anticipated only if water (or hydroxo-) ligands do strongly interact with certain strongly H-binding amino acid side chains in such an unpolar environment (Glu, Asp, Asn, Arg, Ser …).

Chapter 4
The Evolution of Essentiality

"Nothing in biology makes any sense except in the light of evolution" (T. Dobzhansky)

Chapters 2 and 3 dealt with the various aspects of how complex formation properties control the chances of chemical elements to be or become essential, given limiting criteria which are due to (a) reproduction of the entire being and its specific autocatalysts and (b) histological complexity of living being (phenotype heterogeneity). Since there is no known way to reconstruct essentiality patterns with respect to fossil lifeforms as yet, any attempt into an investigation concerning prehistoric essentialities has to start on some combination of

1. Arguments on evolution
2. Comparison of essentiality patterns in different groups of organisms
3. Chemical (biocatalytic) versatility as modified like shown in Tables 1.1 and 2.1

Thus there is a complex interaction of various influences; however, in early phases of chemical and biological evolution: much before specific transporters could have developed, biomass should have taken up metal ions from the environment according to chemical equilibria, providing some distribution pattern from external resources such as seawater which is likely to provide the starting-point for "general" metal essentiality at least. Presumably this array of metals (K, Mg (in metazoa also Ca), Mo (or W), Mn, Fe, Cu, Zn) does correspond to the outcome of the change of complexation (ligand) properties which are caused by principal processes of chemical and prebiotic evolution. Notably, there are very few reactions in prebiotic chemistry which actually depend upon metal ion catalysis or template effects ($Mo(CN)_8^{4-}$-promoted formation of adenine by HCN pentamerization (Follmann 1985)). Even though amino acid yields during energetization of "prebiotic" starting mixtures are considerably increased by metal ion additions, the latter takes place at larger (more realistic and prolific) electromagnetic wavelengths than without metals, producing some additional products like proline (Bahadur et al. 1956) and activation of dinitrogen by soluble (Mo + Fe) systems would account for possible use of N_2 otherwise not (Ferris and Chen 1975; or hardly at best, Kobayashi et al. 1989) feasible. For some comparison of the (usually small) contributions of LMCT-active or -inactive metal ions or -sulfides on photochemical (tungsten lamp) amino acid formation see Santamaria and Fleischmann 1966. Hence it is likely that "advanced" systems of "prebiotic" products would not conserve the primordial metal contents (which are not required to form them!) but rather accumulate them from the environment according to ambient concentrations and complexation equilibria. Their actual "uses" only commenced with or shortly after biogenesis, thereby implied and controlled by the chemical framework and implications of identical reproduction. Corresponding sources could both be seawater or some minerals undergoing photoweathering. Thus some preliminary yet general statements are possible.

4.1 Evolution and Biochemical Catalysis

Generally speaking, the chance to establish autocatalytic cycles during chemical evolution (Orgel 2003) with or without external "selecting" influences is rather small. On the other hand, some (not all of the) similarities between product patterns of "prebiotic simulation" experiments and contemporary biochemistry suggest that the principal biochemical structures kept constant throughout the existence of life on Earth. Hence autocatalytic feedback loops came into existence only later,

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with the three-functions-rule operating from then on throughout biological evolution. The three-functions-rule calls for either strong or critical autocatalytic cycles. In the very beginnings, metal ions or other catalysts in primeval biomass were unlikely to promote a larger number of transformations, which fact in turn rendered the first organisms highly heterotrophic (Lemmon 1970; Schuster 1984; Rauchfuß 2005). Even elements like Mg or Zn had not many more than three functions close to origins of life, and there should have been no selectivity in metal uptake other than that caused by fractionation biased by differing complex formation constants and ambient concentrations. Because, accordingly, instabilities as (possibly) caused by strong cycles turned up only later during evolution, chances to control them got better. Critical cycles, unlike strong ones, do not display "endogeneous" instability (oscillations, clock reactions, spatial patterning, bistability, etc. (Pota and Stedman 1994)) if left (reacting) alone but do so only if made competing with each other or if they be exposed to other kinds of perturbation (Clarke 1980). Even though an early establishment of autocatalytic feedback patterns is unlikely, several authors give good theoretical (Wächtershäuser 1990) and experimental arguments (Ivanov and Slawcheva 1984) for some parts of citrate cycle and glycine transformations to be directly derived from thermochemical processes at solid–liquid or solid–gas interfaces. If there were autocatalytic features in such reaction cycles already (see Le Son et al. (1998) for an autocatalytic contribution of diglycine product in promotion of Cu-mediated peptide formation) or either were introduced by some product increasing efficiency when a metal ion increasing turnover got coordinated from the environment by complexation to (one of) the products, SNA tells us what would happen. Empirically, Mg and three other metal ions (Fe, Mn, Zn) now are involved as "principal agents" in the Krebs cycle whereas transamination of glycinate is accomplished without metal contribution even now. Yet Mn(II)/Al_2O_3 cooperate in glycine pyrolysis to afford a manifold of partly complicated organic products while in absence of metals at 310°C only HCN + CO_2 + H_2 are formed (Dose and Rauchfuß 1975). In this manganese/alumina system, both reactions of/at cyanometallate intermediates and Fischer-Tropsch-type transformations of the H_2/CO_2 byproducts would be unlikely, other than with Fe- or Co-based systems.

Table 1.1 dealt with the distinctive differences between real biochemical "selection" of metal catalysts and that deemed optimal from knowledge on technical chemical catalysis of these very transformations which "persist" even after >3.5 bio. years of biological evolution. This need not imply that evolution is very sluggish in identifying the corresponding optimum, or the system might be highly conservative: functions of apoproteins loaden by different metal ions tend to be rather variable, while the various kingdoms of biology promote the same reaction by fairly different metal ions (e.g. in hydrolytic enzymes).

Concerning substrates or binding partners of the above-mentioned metal ions, the question must be addressed how many amino acids with additional donor side chains, nucleoside bases with similar "out-of-ring" binding sites must be present to correspond to the above multitude; upon slight modulation of conditions, e.g. changing pH or counter-ions, **linkage isomers** (Balahura and Lewis 1976) of nucleoside and nucleotide oligophosphate complexes of, e.g. Zn^{2+} will interconvert (the metal ion moves to another site), thus in biological systems inevitably interfering with control of their data-transmission which is achieved by methylation. Most of these (except of pyrimidines and large heterocyclic amino acids) are produced in prebiotic simulation experiments (see below) in at least trace amounts (Lemmon 1970; Rauchfuß 2005). When considering functional groups, the key exceptions from the product pattern are thioether (dialkylsulfide) and phenol moieties which only arise in exotic setups, either using educts hardly plausible in the palaeogeochemical setting or processes such as flash pyrolysis at T_{max} > 1,300 K which may be encountered next to volcanoes but are unlikely to afford reasonable amounts of products in larger areas. From "real", contemporary ash-gas eruptions, including products of one pyroclastic flow at Kamchatka and the Kuriles, lots of HCN (of course) were isolated (Follmann 1985), producing also some simple non-biogenic amino acids but none of the complicated products of technical flash pyrolysis which arise, e.g. at a W or Pt wire made to glow by electric current (Ruiz-Bermejo et al. 2007).

Now consider geochemical sinks: transition metals, e.g. manganese, may become almost unretrievable for organisms after formation of refractory oxides by oxidation (unless they are reduced again). MnO_2 forms oxide concretions (part of iron pan and "gluing" together particles of sandstone) in B horizons of soils, thereafter acting there as a non-biological redox catalyst which in presence of air oxygen influences speciation of elements like Ce, As, Cr (formation of

arsenate(V), chromate(VI)) and many others while degrading polyphenols also. There are similarities between contemporary and ancient soils (see BIFs) insofar as non-biological element speciation chemistry is concerned. Though MnO_2-dependent/mediated oxidations of, e.g. Cr, As or phenolic compounds do not directly depend on biology, they require elemental oxygen and thus the onset of water-based photosynthesis. The present state of Martian regolith (mainly ferric, but still ferromagnetic and thus not completely oxidized to Fe(III), venting of CH_4 at several sites) suggests retention by Fe(III)-containing oxides be feasible on early Earth also while providing some redox buffer also. For a more reducing early Earth, the variety of reaction will increase in the upper sediment also even before biopoiesis.

4.2 A Three-Step-Model for Uptake and Functionalization of Metal Ions Enforced by Chemical Evolution Itself (Bootstrap)

As was shown before, it is an erroneous assumption that systems (chemical entities) which act as efficient catalysts on other species are given to work in the same manner if integrated into some autocatalytic system. Yet, with autocatalysis – at least as capability for reproduction – being a crucial part of any meaningful definition of life, the rule of three functions must have applied to the most early organisms already quite like it does control essentiality today, regardless of their exact chemical composition.

Up to now, this work was restricted to processes which occurred in the framework of biology, that is, after the origins of life on Earth. This both concerned problems of dynamic stability and of enrichment/"functionalization" of chemical elements (mainly metals), including consequences of integration into larger, more differentiated organisms. This was achieved using Eq. 2.4 to construct some mapping of essentiality, toxicity and additional biochemical features on the c/x-plane. This mapping distinguishes some small subplane in which essential elements gather from all the others. All recently known organisms use a multitude of metals ions to promote or accomplish (parts of their) enzymatic functions (Mg, Mn, Cu, Zn, most often also Fe, Mo), hence it is reasonable to assume this will hold for all the times since onset of biological evolution, that is, ever since biogenesis. This is in stark contrast to the small number of processes in chemical evolution which involve metal ions: the latter is very small regardless of the specific ion and chemical starting conditions (cp. Fränzle and Markert 2002b; Lemmon 1970; Evard and Schrodetzki 1976). For example, the proteinogenic amino acids, possibly except proline (Bahadur et al. 1956), can be made without adding metal ions to the "prebiotic" solution, as can be purine and pyrimidine bases. Sugars or sugar phosphates are formed from HCHO (+glycol aldehyde) or aldol phosphates with arbitrary bases such as NaOH (Eschenmoser 1991), also without that catalysis by clay minerals, alkaline earth or Pb^{2+} ions needed in the classical formose reaction.

There is only one way to reconcile both statements, that all living beings need metal ions to sustain enzyme activities and thus life while chemical evolution could proceed in their absence also, namely, to invoke some spontaneous enrichment of various metal ions in what was to become biomass shortly before or directly in the transfer from chemical to biological evolution, that is, closely before biogenesis took place. Even though this process involves species which are hardly soluble in water or/and undergo strong adsorption to clay minerals, this need not imply that primeval organisms were most tightly associated with mineral interfaces or even partly consisted of them. However, the introduction of metal ions and their functions into early biology might have been akin to heterogeneous catalysis, given the corresponding capabilities of metal-exchanged clay minerals. This process most likely was due to coordination chemistry going on in and on the material subject to chemical evolution. The latter process gradually and steadily altered ligand properties of the substrates of chemical evolution, starting with simple gases and ions and thereby producing amino acids, N-heterocycles or phosphated compounds which are far more efficient ligands. It is hypothesized that implications of Eq. 2.4 and the corresponding changes of the electrochemical ligand parameters can show how and why ions like divalent Mg, Mn, Cu or Zn are preferably bound to biomass with respect to other metals which (thus?) attained less or no biochemical significance.

This model of events is detailed into some three-step-scenario for better analysis and understanding. It is based on experiments and reasonings by Beck and Ling (1977) and Kobayashi and Ponnamperuma

(1985a,b), linking thoughts on "best" or most probable ligands and shifts of prebiotic ligand yields by metal ion additions to the c/x approach. However, it should be noted that, although many limiting conditions are described in the present work which contrive plausible scenarios, this *gedankenexperiment* detailing three steps into biopoiesis and the (probably non-steadily) changing role of metal ions in these processes cannot yet be taken to provide one single complete picture. One reason for this is that the set of data is fairly small and somewhat focussed on metal ions known to be essential, and, in addition, some of these data for metal ions are analyzed with respect to oxidation states (e.g., Fe(III), Cu(II)) the relevance of which to early Earth is doubtful even given a CO_2-dominated atmosphere and the fact that certain metal-dependent "prebiotic" reactions were induced using these very ions (Bahadur 1954; Bahadur et al. 1956 (both dealing with Fe); Huang Le Son et al. 1998 (on Cu)). Yet, it is possible to depict chemical evolution in three main steps which – besides other effects – will convert non-metal compounds of low or non-existent metal affinities into precursors of biopolymers which strongly attract certain metal ions but not all. This permits to reconstruct the compositions of early atmosphere and the kind(s) of metal sources/concentration levels of mineral salts next to the eobionts likewise, to some detail. For example, reliable conclusions include

1. That REE ions could not be effectively absorbed by protobiomass, excluding, e.g. contributions of Ce to general and of Eu to photoredox catalysis (which otherwise might have enabled some kind of primitive, "semi-inorganic" photosynthesis, and otherwise capable of activating sugars as well as amino acids and their phosphates into CO_2 binding and other reactions), whereas
2. There is or was just some narrow pH interval which permitted nowadays essential elements to remain dissolved in water up to levels which made them all accessible in step 2. For example,)recent seawater provides plenty of Mg or Ca but only minute traces (pico- to lower nanomolar amounts; Nozaki 1997) of Fe, Mn, Cu or Zn whereas modern freshwater contains "adequate" Fe and Mo but too little of the other metals, especially weakly complexing elements to have them bound to precipitates of insoluble salts of simple ligands.
3. Complexation in solution rather than on some interface (sorbate, precipitate, polymer) is unlikely to increase metal availability at "exotic" pH values given the small dynamic equilibrium concentrations of even small-molecule (C_2- or C_3-)-chelators like glycinate, oxalate, malonate in seawater. In fact, it would even pose an obstacle against "progress" in(to) steps 2 and 3, much like the problems of contemporary plants or fungi to extract metals from some soil highly loaded with chelating agents like humic acids, other polyphenols or amino acids.

Much like in Section 2.2.4, an equilibrium between mono- and bidentate binding to some (mixture of getting more) complicated ligand(s) is assumed, for which some critical $E_L(L)$ applies, with variation over time as an additional feature now: we no longer deal with linkage isomerism or variable occupation of several sites in some chemically homogeneous ligand system which stays unaltered in time but now ask what will happen due to chemical transformations which create or alter ligand sites with time going on from step 1 to 3. Passage of metals from "primitive", i.e., monodentate precursors into bidentate binding to polymer or colloid phases is supported by an increase in c (second column in Table 4.1) while x can change in different ways. The difference between x_{1d} and x_{2d} for a given metal ion dictates whether this metal ion will or will not be taken along with the polymer; in cases of very similar values (e.g., Mn(II) or Ce(III)) passage will occur due to larger c regardless of the kinds of ligands. However, according to Eq. 2.4 certain complex formation constants are related to possible equilibrium states, and thus it must be seen whether these equilibria permit uptake of metal ions at all:

$$\Delta E_L(L) = \Delta c_{1;2} / (x_{2d} - x_{1d}) \qquad (4.1)$$

then (2.4)

Enrichment of Co(II) by early polymers thus would occur at $E_L(L) \geq -0.47$ V, that is, for almost all biologically relevant ligands other than thiolates. In considerable contrast with this, amino acids have $E_L(L) \approx -0.05$ V while the corresponding value for peptides is about zero, oligocarboxylates ranging from about −0.13 V (malate) down to some −0.26 V (citrate). The difference in $E_L(L)$ caused by chemical evolution thus will suffice to promote step 1 **if** the absolute value of $E_L(L)$ of the educts is not higher than some +0.2 V – provided

Table 4.1 On the possibility to extract metal ions from aqueous media by a mixture of proteinoids or/and solid alkaline earth carboxylates or aminocarboxylates, representing the state of matters after step 2. "Required" minimal differences of electrochemical ligand parameters (column 5) were calculated using Eq. 4.1

Metal ion	$\Delta c_{1,2}$	x_{1d}	x_{2d}	$\Delta E_L(L)$	Log k_0
Mg(II)	+4.04	−4.6	8.24	−0.31	1.35
Al(III)	+12.68	−17.22	41	−0.22	2.97
VO^{2+} [b]	+6.95	−7.91	(14.17)	−0.31	1.99
Mn(II)	+2.81	−5.2	−5.32	[a]	[a]
Co(II)	+4.31	−5.3	3.93	−0.47	3.90
Cu(II)[b]	+7.34	−8.0	21.84	−0.25	3.67
Zn(II)	+5.44	−8.93	8.69	−0.31	2.47
Cd(II)	+3.6	−5.25	5.7	−0.33	2.43
Y(III)	+5.71	−11.6	−11.03	[a]	[a]
La(III)	+3.21	−9.48	−17.58	+ 0.40	(−3.96)[c]
Pr(III)	+3.65	−9.48	−14.58	+ 0.72	(−6.66)[c]
Cr(III)	+9.54	(−15.4)	(13.9)	−0.32	4.45
Fe(III)[b]	+13.71	−22.42	21.39	−0.31	4.24
UO_2^{2+}	+9.98	−14.35	15.86	−0.33	3.92

[a]There cannot be calculated any meaningful value for difference of $E_L(L)$ because differences between values for x_{1d} and x_{2d} are (too) small

[b]This ion can only exist/persist in an oxidizing environment. *Banded iron formations*, the oldest of which are more than 3 bio. years old, do suggest that "environmental" conditions (i.e., ambient redox potentials) were sufficiently oxidizing to produce and sustain solid Fe(III)(-containing) oxides already during or soon after biogenesis. Whereas BIFs may be produced in laboratory by intermittent photooxidation of dissolved organic matter (DOM) by Fe(III) is much more efficient ($\Phi \approx 0.2$–0.9 at $\lambda >$ 300 nm) than photochemical hydrogen production using Fe(II) ($\Phi \leq 0.05$ at $\lambda > 300$ nm) (regardless of ligands, additives or pH, cp. Horvath and Stevenson 1992). Thus, in the photochemical dynamic equilibrium state which is produced by UV–VIS irradiation Fe(II) prevails even under the recent atmosphere when C/Fe >> 1 like in common freshwaters (typical DOM levels being several mg/L (some 50 μmol C) while $C_{Fe} < 10$ μmol/L)

[c]As neither mono- nor bidentate complexation takes place with $-\log k_{diss} << 0$, there was no enrichment of La^{3+}, Pr^{3+} or similar ions in going-to-be biomass during chemical evolution

they do behave as ligands at all. This does hold for all anionic ligands regardless of the atoms forming their donor sites while neutral S-, C- (CO, C_2H_4, C_2H_2 or isocyanides), P-, As- and unsaturated neutral N-donors (e.g., nitriles), surpass this value, often considerably.

If components of the primitive atmosphere to be integrated into ligands do not act as ligands at all, step 1 will be realized in any case; conversely, however, substantial amounts of CO ($E_L(L) = +0.99$ V) in that atmosphere would have impeded step 1 exactly since it is readily used in "prebiotic" reactions (e.g. Miller and Schlesinger 1984; Kobayashi et al. 1989). Leaving aside the geochemical difficulties with assuming such an atmosphere, some extremely reducing atmosphere (i.e., one dominated by CH_4) would produce – even more than and before amino acids – alkenes and alkynes (Owen 1987; Wayne 1991; Kasting 1993), both neutral ligands being distinguished by very high $E_L(L)$ values. The gravitational attraction of Earth is too weak to retain any larger quantity of dihydrogen which could be formed, e.g. by photooxidation of dissolved Fe(II) or vented by certain volcanoes. Hence, the model experiments combining CO or CO_2 with larger concentrations of H_2 are hardly realistic. Methane, on the other hand, is stably kept in the atmosphere but photolabile. These reactions, however, would not stop the transformation sequences as either does undergo rapid consecutive reactions:

- Alkenes would polymerize to macromolecular hydrocarbons which do no longer coordinate.
- Alkynes are hydrated to yield aldehydes, the latter
- Subsequently producing either amino acids by the Strecker reaction forming aminonitriles first (Eschenmoser 1991) or
- Undergo photoassisted oxidations to yield carboxylate (ligands) or
- Become integrated by cyclotrimerization into benzenoid hydrocarbons or pyridines by reacting with each other or nitriles (including HCN), respectively.

Given this, no negative (impeding) effect on binding and activation of metal ions in the reaction mixture would be anticipated, which agrees with empirical results obtained by Kobayashi and Ponnamperuma (1985b). The latter reaction was initiated by some spark discharge acting on methane, ammonia and water including several metal ions. The mixture yielded substantial amounts of ethane (a non-ligand) and ethyne, propyne, also benzene, but only traces of CO while the original pH of the solution was 8.0 and later increased to 8.7 regardless of metal addition even though NH_3 was consumed and acids produced. There apparently is an unaccounted production of larger amounts of Brønsted bases although NH_3 is consumed, net oxidation takes place and carboxylic acids are formed. Addition of a mixture of metal ions was to increase amino acid yields by some factor of 5, ending up at pH 8.7 also (ibid).

The small complex formation constants $-\log k_{diss}$ with metals like Mg, Fe(II) or Cu(II) suggest that either

not all the recently essential elements were so from biogenesis onwards or thereafter some change of this average value towards −0.32 V occurred, entailing establishment of biocatalytic functions for several (other) metals which hitherto were enriched in prebiotic solid phases (see below) already without having acquired corresponding functions so far. Thus, while the "classical" primeval soup would even produce problems in metal-ion acquisition, these counter-arguments would not hold for a (possibly photoassisted) synthesis on some mineral interface, which was demonstrated for amino- and polycarboxylic acids before (Dunn et al. (1981); photoamination of glycolic and lactic acids on CdS or of nitriles on ZnS/CdS (Gärtner et al. 2007)), in the prebiotic setting to be followed by corrosion of the latter much in the same way recent cryptoendolithic organisms do. Then the mineral covered by the forming proto-biomass would become the required resource of metal ions itself, with the organic film or shroud controlling loss to some liquid phase efficiently. Eventually, the organic cover would separate from the corroding support, thereby forming protocell structures.

If there was such a kind of "average biomass $E_L(L)$ degression" upon chemical evolution into early biology (i.e., conversely to that change shown for aging soil in Fig. 2.23), it was probably linked to spreading functions of phosphorus substituents in developing and differentiating biochemistry (the present author does not "believe" in anything like a "RNA world" preceding or existing parallel to biogenesis). P-oxo species (besides thiolates) are distinguished by very low $E_L(L)$ values, with protogenetic storage functions and possibly also energy storage effected by other than P-based oligo- or polymers in the beginnings even though phosphorylating agents can be produced in realistic prebiotic circumstances (Saygin 1984). In the latter case, there is **photochemical energy storage** consuming cyanide and electrons to produce polyphosphates via carbamoylphosphate which latter is a versatile condensation agent itself:

$$\begin{aligned} &2\left[\mathrm{Fe(CN)_6}\right]^{3-} + \mathrm{CN^-} + \mathrm{PO_4^{3-}} + \mathrm{H_2O};\\ &h\nu \rightarrow \mathrm{H_2N-COOPO_3^{2-}} + 2\left[\mathrm{Fe(CN)_6}\right]^{4-} \end{aligned} \tag{4.2}$$

$$\begin{aligned} &\mathrm{H_2N-COOPO_3^{2-}} + \mathrm{HPO_4^{2-}} \left\{\mathrm{M^{2+}}\right\}\\ &\rightarrow \mathrm{P_2O_7^{4-}} + \mathrm{NH_3} + \mathrm{CO_2};\\ &\mathrm{M = Be, Mg, Ca \text{ or } Sr} \end{aligned} \tag{4.3}$$

During the later steps of chemical evolution with metal ions already bound to the material by chelating ligands which are mutually interconverted, the sets of lines in Fig. 2.15 can be applied likewise, providing Fig. 4.1. Figure 4.1 shows that chelating ligands are more strongly bound to most metal ions than intermediates like nitrate, nitrite or cyanide, although their electrochemical ligand parameters tend to be considerably lower. Thus, chemical evolution promotes uptake of metal ions due to the "chelate effect" except for accumulation of CO or alkenes trapping intermediates as carbonyl or olefin complexes.

Generally speaking, biopoiesis on Earth (or maybe on some adjacent celestial body in the solar system from which it got to here) is/was much more than a fundamental emergent process (*sensu* Müller-Herold 1984), but also associated with a remarkable change in structural aspects: metal ions, hitherto of minor significance, except for controlling selectivities of certain reactions, suddenly turned into indispensable catalysts upon biogenesis. Probably in the very beginning only few among the metals Mg, Mn, Fe, Cu, Mo or Zn were used, yet chemical evolution itself produced solid phases capable to separate these from, e.g. Cd, Al or Co and start biological evolution without actively involving the latter (which holds for Al up to this day).

There is earlier work on the relationship between complexity of autocatalyst assemblies and the chance of their spontaneous origins (King 1982); e.g. for a kinetic complexity of Y = 12 (12 components which are to interact for onset of autocatalysis/reproduction), the probability of a spontaneous onset of autocatalysis is about 10^{-9} (King 1982). Given that this likelihood rapidly tends towards zero for larger sets of compounds, kinetics and autocatalysts (e.g. $P = 2 \times 10^{-33}$ for Y = 30) whereas parallel use of quite a number of metals besides ≥ 5 non-metals in an autocatalytic manner (which is not easy to achieve under such circumstances) marks biogenesis (if it is not even tantamount to it), it comes to no surprise that most principal biochemical reactions are closely related to each other, comprising the primary reactions of organic carbonyl compounds ((thio-)ester-, aldol- and Knoevenagel condensations, ester and amide formations) besides preparation of effective leaving groups (i.e. polyphosphates) and their replacement by most diverse nucleophiles, whereas organometal chemistry is almost omitted. This suggests that, although a number of different metal ions

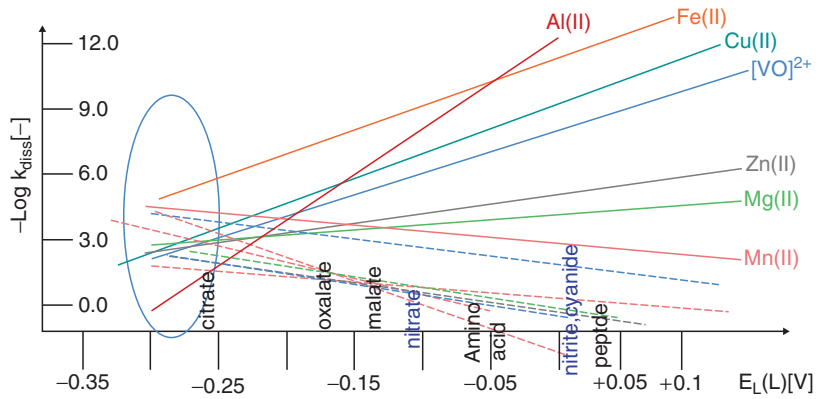

Fig. 4.1 Relative stabilities of complexes with monodentate (*blue letters*) and chelating (*black letters*) ligands with seven different metal ions. Bidentate complexation is shown by straight, monodentate by broken lines, identical line shadings refer to the same metal ion. Compare Table 4.1 for the intersection points with the engulfed area between some –0.25 and –0.32 V for Mg, Cu, Zn or Fe(III); the value for Al(III) is higher (–0.22 V) while for Mn(II) no "realistic" intersection point does exist. Ongoing chemical evolution supports metal fixation to developing biomasses to an extent increasing with both time and evolution as recombination of fragments produced by photo- or radiochemistry (once again supported by certain metal ions), accompanied by thermal condensation (e.g. Lemmon 1970; Ivanov and Slawcheva 1984; Rauchfuß 2005) afford products with more efficient donor behaviours towards metal ions

was employed already then to catalyze protobiotic reactions, the number of different kinds of organic reactions had to be small; in addition, high-yield products of primary and early steps of prebiotic transformations include such compounds (besides formic, acetic, glycolic and simple amino acids), mainly HCHO, CH_3CHO, glycol aldehyde, acetone and glyoxyllate ion, nitriles such as CH_2=C(NH_2)CN (precursor, e.g. to serine; Eschenmoser 1991), and sugars derived therefrom, making them logical substrates and the corresponding set of transformations – closely related to each other – the logical starting-point of biochemistry, regardless whether and by which species (including organics) they can be catalyzed. Yet, as similar as these reactions are formally (Sykes 1977), appropriate catalysts (if any) differ considerably among them, giving rise to the above situation during biogenesis.

Insofar metal activities are required for both inheritance and "ribozyme" functions, and RNA will enrich but Zn and Mg upon its formation (which apparently is not a conceivable part of chemical – rather than biological – evolution), this provides an additional argument against any kind of "RNA-world" which would rely upon no additional kinds of polymers. Instead, peptides or even proteins would be required to provide the very metal ions needed to activate ribozymes by active sequestration and transport. This suggests – though does not imply – that RNA-related catalytic functions, if they and RNA were already at hand – were not required in turnover of materials around biopoiesis. Mg and Zn are not really (ultra-)trace elements in biology, their large numbers of functions calling for substantial enrichment regardless of the kind of organism. As suggested before, the same criteria of selective – yet comprehensive with respect to universally essential elements other than K – enrichment from a resource by chemical evolution turning over C, H, N, S compounds into amino acids etc. will also hold when the resource is a *solid* support. Examples advanced in the literature sometimes even go beyond the idea of some solid ion source into speculations that the eobiont be some "blend" of clay minerals and organic compounds, with "genetic take-over" taking place only much later (Cairns-Smith 1982), or Wächtershäusers (1990) idea that formation of pyrite released trace admixtures of metal ions besides forming organics. Then, however, it must be taken into account that sulfide minerals as a metal ion source can hardly reproduce the distribution of metal ions and the essentiality pattern observed in bioinorganic chemistry, as hardly as seawater but unlike carbonate interfaces. Affinities of ligands thus produced, however, were too

low to sequester more than trace amounts of Mn, Fe, Cu or Zn from media (solutions) akin to recent ocean water (cp. Fränzle and Markert 2002b).

Another important question is still open: is there any explanation for the "discrepancies" in use pattern of metal ion catalysts collected in Table 4.1. Conceivably geochemical situations during biopoiesis or later changes might cause other metals to be used, with the formal choice "frozen" to some extent, the more if activation of the substrate would not succeed in these chemical conditions. Extending this argument to possible redox reactions in astrobiology, reduced V (or Ti(III)) can even reduce otherwise kinetically inert ClO_4^- (besides of NO_3^-) which was recently detected in Martian soil. Of course perchlorate would have piled up after possible Martian life went extinct but it would not have precluded it, rather it (besides nitrate) could act as a versatile terminal electron acceptor. Concerning V, Cu and Mo, several of their metalloenzymes would have been of no use whatsoever in an anoxic atmos- and hydrosphere:

- Superoxide dismutase (Cu + Mn or Cu + Zn)
- Haloperoxidases (V or Fe/porphyrine (haem)) could have used only traces of H_2O_2 in rainwater in an anoxic atmosphere (although the aq. H_2O_2 level was possibly higher for lack of an ozone layer then)
- When there were not yet metazoans, means of intercell communication effected by NO or alkenes (C_2H_4 as a plant hormone) binding to Cu(I) were almost irrelevant

Thus omitting NO-, O_2-, H_2O_2-processing enzymes including haloperoxidases from Table 4.1, all of which would work best with either V, Mo, Co or Cu, there are few "discrepancies" left, namely

1. Hydrolyses of functionalized phosphates (NTPs, nucleic acids) and
2. Of guanidines (arginine degradation, urea cycle)
3. Polycondensation of amino acids and
4. Oxidations of methyl groups and of CO (CO dehydrogenase), plus possibly (if Ni is involved)
5. Transformation of CO into (thio)acetate like in Monsanto synthesis of acetic acid or its esters

This is to say that (almost all of) these presumably ancient biochemical transformations either attended "optimum" secondarily for sufficient time has passed since their becoming involved in biology or else there was some "match" with the primeval selective extraction or retention protocols of metal ions already due to selective uptake in the evolving polymeric organic matter. Reactions 1–3 at least were important already much before the Precambrian oxygen disaster, with phosphate ester hydrolysis promoted by Zn^{2+}-supported proteinoids and polycondensation of amino acids by Cu^{2+} and peptides in a thus autocatalytic fashion, hence presumably predating biogenesis. In "real" biochemistry, yet, Cu or Co^{2+} are omitted in all these reactions in favor of Mn, Zn or Mg. One explanation (Williams and Frausto Da Silva 1996) casts doubt on access to Cu in still anoxic conditions, with Mn (forming a very easily soluble sulfide only), Zn or Mg, not undergoing any redox reactions, were not touched by the chemical conditions then. While both Fe(III) and Cu(II) often are more efficient redox mediators, they or oxidants required to make them did not yet exist in larger amounts. The case is less clear for V, Mo, allowing for thioanion formations (Pourbaix diagrams in Kaim and Schwederski 1993; Coleman 2003). Omitting the metals for which either retrieval or chemical activation were difficult then, most of the "discrepancies" resolve, while the prominent role of molybdenum in biological redox catalysis can be readily understood: Mo (+Fe) does promote reductions of N_2 and NO_3^- (which is formed photochemically in an anoxic but CO_2-containing atmosphere also), oxidations of sulfite, dialkylsulfides or aldehydes (aldoses) quite readily. Though hydride abstraction or disproportionation of aldehydes can also be effected by Al or Zr (Sykes 1977), versatility is too small to fulfill the three-functions-rule while vanadium does undergo such reactions in organometal species like vanadocene only (Floriani 1983; Elschenbroich and Salzer 1988). However – a fact as strange as the total omission of forming chiral and chirogenic metalloenzyme catalysts by using six different amino acid side chains around the central ion – organometal species other than those of Co are very rare in biology (Williams 1986), except, probably for sake of detoxification, as $E(CH_3)_x$ or $EO(CH_3)_x$ biomethylation products (terminal metabolites, to be released rather than recycled for all E ≠ As (which is not a metal; Irgolic 1986)).

Accordingly, one should assume that the crucial emergent process (i.e., biopoiesis) rather took place in surrounding waters enriched with mineral salts, possibly acidic solutions, e.g. mineral springs degrading nearby carbonates or ponds slowly drying out, than in the open ocean. The geochemical plausibility and implications will be discussed next.

4.2.1 Fractionation of Chemical Elements in and by Polymeric Antecessors of Biomass During Chemical Evolution

It should be pointed out that in many corresponding experiments joint, unresolved effects of a multitude of added metal ions – usually meant to mimic seawater – were investigated, rather than checking catalytic or directing effects of one single additive at a time. Possibly in such mixtures, realistic as they might appear, certain contributions of the above kinds to chemical evolution even would cancel each other. Nevertheless, the scenario outlined here has to address multiple effects without attributing them to certain ions generally. In the above simulation experiments, some (geochemically more or less plausible) mixture of speciation forms containing at least the biologically most significant non-metals (C, H, N, O, often S) in aqueous solution or gas phases is exposed to energetization: corresponding experiments cover the entire range of chemically activating energy sources except of electrochemistry and ultrasound, namely heat, UV radiation, γ-radiation, beams of charged particles (e, p, α), shock waves, spark- or glow discharges, photoelectrochemistry etc. Often, but not always, the above elements are used in their reduced forms and in some cases pertinent chemical transformations were investigated after addition of metals like Fe (Bahadur 1954; Ruiz-Bermejo et al. 2007), Mn (see below), Mo (Bahadur et al. 1958), Cu (Beck et al. 1977; Huang et al. 1998) or of some mixture simulating seawater (Kobayashi and Ponnamperuma 1985b; Sakurai and Yanagawa 1984). There are many such experiments in the literature which combine species which are unstable besides each other owing to thermodynamics, e.g. HCHO or its oligomers and nitrate or CO and nitrite, allowing for spontaneous or photoassisted (Bahadur 1954) redox reactions some of which afford corresponding products in a downhill manner. Yet it is hard to assume that there should be prolific sites for such reactions on early Earth except, perhaps, for erosion of Fe(II)-containing minerals and for sites of large T differences ("black smokers", fumaroles) although the idea that "downhill" processes might provide reduction equivalents for prebiotic synthesis took some hold in the literature (e.g., Freund 1984; Wächtershäuser 1990). Solid interfaces of other kinds were also considered (Chen and Chen 2005).

In the latter cases, individual contributions by metal ions possibly interacting with some intermediates can no longer be distinguished. Generally speaking, such experiments afford both amino acids and other biologically relevant substances albeit in modest to very low yields (e.g. ≤ 2% of carbon reservoirs are turned into glycine), the best yields being obtained by shock waves (≈20 nmol/J of total amino acids; Schlesinger and Miller 1984) or beams of high-energy charged particles (Kobayashi et al. 1989, however using CO in the gas mixture). Unlike UV radiation, shock waves are unlikely to induce "inverse" reactions given the presence of metal ions, e.g. prolific formation of (then decomposing) prebiotic ligand radicals or reactions with OH whereas either metal interfaces, solid salts or complexes undergo strong and efficient alterations if exposed to ionizing radiation (Bjalobženskij 1971; Stiller 1987). Some thermal transformations of "prebiotic" mixtures or intermediates may be enhanced and directed by metal additions, e.g. thermolysis of solid glycine at 240°C with additions of $MnCO_3$ and alumina (Ivanov and Slawcheva 1984) which affords a manifold of carboxylic (formic through heptanoic) acids, higher amino acids up to 2,4-diaminohexanedioic acid and many aromatic compounds also.

When concerning the model of the process (ongoing interaction between developing products of chemical evolution and ambient metal ions), the crucial question will be whether possible selectivities of the developing materials could bring about enrichment of those very metal ions which now are essential. Generally speaking, in chemical evolution non- or weakly binding ligands (CH_4, CO_2, monodentate ions or molecules like CO, NO_x^- or Cl^-) from the early environment are turned by photochemical or other energy-providing processes into others which either are still monodentate but strongly binding (e.g. cyanide) or chelators (amino acids, dicarboxylic acids, heterocyclic compounds) which are mostly bi- or tridentate, hence former stronger complexes. However,

1. c and x for n-dentate binding are not just n times the values for "simple", monodentate ligands (some kind of numerical representation of the "chelate effect"), thus selectivity of metal trapping will change during this process.
2. There is no simple trend concerning the change of electrochemical ligand parameters throughout chemical evolution (unlike the trend observed upon ageing/"ripening" of soil (Fränzle 2008a)).

So, while chemical evolution does change metal affinities of the going-to-be biomass both absolutely and

relatively among the metal ions, with some trend towards stronger complexation, understanding of details and of their sequence warrants some more precise analysis. With metal ions being redistributed between water and solid associations of substances going to become biochemically relevant, it is to be investigated whether such spontaneous associations, including Mg and Ca salts of fatty acids capable of occluding amino acids as well as "genuine" polymers, will (selectively) enrich (which of the?) later-on essential elements.

Among the ligands which were considered as preferential metal binding partners during chemical evolution in older literature, one is omitted here although it was included as an educt in all the classical Miller-Urey (1953) experiment, that done by Kobayashi and Ponnamperuma (1985a, b) or the semitheoretical analysis by Beck and Ling (1977): that ligand is **ammonia**. However, ammonia is most sensitive towards UV decomposition, and only traces of it are reproduced from other N-compounds in geochemically realistic conditions. In addition, from N_2 (as a much more realistic alternative) nitrogen will not be introduced into, e.g. amino acids, nitriles or amines (Ferris and Chen 1975) after activation attempts by either photochemistry or shock waves. This problem can be overcome by using ionizing radiation or by heterogeneous catalysis (Bahadur et al. 1958): dinitrogen is fixed into amino acids during photolysis of formaldehyde solutions at solid MoO_3. Presumably lower oxidation states of Mo ($Mo^{<VI}$) will form which can reduce dissolved N_2 when traces of iron and sulphur are also present. Apart from this, the only realistic primordial N sources would be nitrogen compounds which form by shock waves (lightning) during thunderstorms in the atmosphere, mainly NO and HCN (Kasting 1993) and the more persistent of their hydrolysis products and those of addition reactions in the ocean. These include nitrite, 2-hydroxinitriles (which form with HCN in reducing atmospheres (Schwartz and Voet 1984)), with $E_L(L)$ close to that of other nitrile ligands (some +0.35 V), and possibly simple oximes which form by NO trapping of simple organic radicals formed during photochemical reactions ($E_L(L) \approx 0$ V), e.g. along the following sequence of reactions (4.4) through (4.8):

$$\mathrm{R-COO^- + M^{n+} \rightarrow [M(R-COO)]^{(n-1)+}} \quad (\mathrm{M = Fe(III);\ Cu(II)\ etc.}) \tag{4.4}$$

$$\mathrm{[M(R\text{-}COO)]^{(n-1)+}\ (h\nu;\ -CO_2) \rightarrow R + M^{(n-1)+}} \tag{4.5}$$

$$\mathrm{R + NO \rightarrow R'CH_2\text{-}NO,\ if\ R = CH_3\text{:}\ CH_3NO\ (R' = H)} \tag{4.6}$$

$$\mathrm{R'\ CH2\text{-}NO \rightarrow R'CH{=}N(OH)\ [tautomerization]} \tag{4.7}$$

and

$$\mathrm{CH_2{=}N(OH)\ \{V\ or\ Mo\ complexes\} \rightarrow}\ \text{glycine (even at −20°C!), hydantoine, sarcosine, N-carbamoylglycine, oligomers (Hatanaka \& Egami 1977)} \tag{4.8}$$

At low carboxylate and/or iron(III) concentrations the photo-Kolbe-dimerization or H transfer among alkyl radicals formed using near UV and Fe(III) becomes much less likely (very low yields) than quenching by the metastable NO radical in manner (4.6). For example, irradiation of Fe(III) propionato complexes, usually producing rather ethane, ethylene and (traces of) butane at $C_{C2H5COO^-} \approx 0.1$ M (Grikos and Hennig 1988), will provide acetaldoxime via nitrosoethane when there are low organics concentration levels but some NO, regardless of UV flux densities. After all, the process which introduces metal ions into biomass upon chemical evolution consists of three steps:

1. Transformation of substances which do not act as ligands into mono- or bidentate species, having electrochemical ligand parameters around 0 V, except for oligocarboxylates (between −0.3 and −0.15 V) and gases CO, NO ($E_L(L) \geq +1.0$ V), namely amino acids, carboxamides and cyanide, if NO (made by lightning bolts in reasonable yields (Kasting 1993; Wayne 1991)) is available and transformed into nitrosoorganic intermediates by alkyl radical quenching, also nitrite and oximes are at hand (which also are monodentate ligands with $E_L(L)$ about 0 V).
2. Precipitation of amino- and longer-chain fatty and dicarboxylic acids by alkaline earth ions or Zn^{2+} etc., sometimes forming membranes (much like Pfeiffer's cell) or pseudocellular structures ("microspheres" (Dose and Rauchfuß 1975); "marigranules" (Yanagawa and Ogawa 1984)). In this second phase, spontaneous formation of both polymers and self-organized compartments occur. The latter might start with mixtures containing glycine, acidic and aromatic amino acids and take place in hot, acidified ocean water enriched with all Mg, Ca and transition metal salts.
3. (Additional) Uptake of heavy- and light metal ions from the solution (sea- or freshwater, possible

peculiar hydrochemical areas such as mesothermal marine mineral springs) by the precipitate or membrane, which thereby attends catalytic functions. In turn, some of these newly introduced catalytic features influence and alter products from steps 1 and 2, e.g. when oxoanions of Mo or V are attached to the membrane (under different conditions given the differences in x for various oxidation states of V and Mo, respectively), step 3 of the above scheme will occur. This produces more ligands which, like oximes and hydantoines, are capable of enriching V and in the same turn provide a backbone or template, blueprint and condensation agent (hydantoine, carbamoylglycine) for peptide-like polymer formations. Anyway, monodentate (NO, nitrite, oximes) N-donors are converted into bidentate ones.

This sequence of events (notwithstanding some overlaps among the steps, of course) should give rise to a **primordial BSE**, with the protobiont/eobiont or "last common ancestor" producing some assembly of metal ions with corresponding concentration factors from the environment. The composition of that assembly will "favorably" differ – considering the staunch change in the roles of metal ions upon biogenesis – from those patterns seen in competitor assemblies which not (yet) "learned" how to reproduce. Although there are reactions which can introduce N into the monomers later to become parts of biopolymers (see above and below), the very high C/N ratios of even contemporary organisms in strongly reducing biota (which is likely to be dictated by biochemical thermodynamics also; Eq. 2.14) raises some question: if there had been a very reducing paleo-Earth atmosphere and hydrosphere, C/N in first organisms likewise should have been close to infinite. But what, then, for the principal role of N-organics in all formation of protein biopolymers, in inheritance and in formation of catalytic (and/or chiral) metal complexes?

4.3 The Three-Functions-Rule as a Controlling Factor in the Origins of Essentiality

The three-functions-rule was derived from a most general argument by SNA on the relationship between autocatalytic orders and summations of "loss" processes in organisms. If, now, three functions must be established synchronously – except perhaps for elements very abundant in biomass without yet having acquired any function(s) – it must be explained how any chemical elements ever could become essential when three functions had to be met at once from the very beginnings of essentiality. At first glance, it appears most unlikely that such an acquisition of triplicate functions would ever occur, but in de Duve's terms this event which obviously occurred multiply cannot be counted among the "events (implied in biogenesis) which are so unlikely that they must be considered a miracle"; hence we must find a reasonable setting for this to happen. This will be outlined afterwards (this is not to suggest most trace elements might be too scarce to fulfill this condition): as a rule of thumb, one biocatalytic function translates into about *one million* atoms of the essential element per cell in man; so, at 1–12 (Ni in urease) metal atoms per enzyme molecule, there always are of order 10^5 copies of a certain biocatalyst molecule per cell, giving a quantitative idea of genetic induction. Conversely rather abundant elements reach fairly high concentrations in biomass (Pb, Al, Ti and others) but would not attend biocatalytic functions if chemical "versatility" in terms of c and x is not appropriate: atoms are so small that the also rather small size of cells does not matter.

In addition, it is obvious from differences concerning essential elements among the large groups of metazoa (algae, fungi, higher green plants, eventually animals) which originated during the large radiation of eucaryota between 1.55 and 1.3 bio. years BP (Feng et al. 1997) that differences among these and relative to bacteria and archaea are large and could occur still fairly lately, especially with respect to animals. Accordingly, adaptive radiation also allows (did allow) for introduction of "novel essential" elements at appreciable rates even though three functions each are required from the very beginnings. Probable reasons include enantioselectivity of biocatalysis, substitution processes which occur in sets of functionally equivalent metalloproteins and the fact that there are metalloproteins which promote several different reactions, also or particularly after undergoing small mutations of the sequence.

The next subchapter is to show how formation of would-be bioligands during chemical evolution and changes of limiting geochemical conditions interacted to bind some "basic set" of going-to-be-essential elements to biomass, with the patterns of essential elements

changing and extending by formation of eucaryota and metazoa. The organelles of eucaryota most likely were independent organisms before, with probably somewhat different patterns of essentiality. Thus, combination of these formerly independent beings into some obligate symbiosis and eventually one joint cell increased the number of essential elements. Remains of this can still be observed today: although other organisms than eucaryota and the antecessors of mitochondria also require manganese, most Mn-dependent enzymes in man, animals and fungi are located in mitochondria only. Hence, conceivably, eucaryotic cells were "constructed" from mitochondrial antecessors and – now extinct – beings which could do without Mn. When heterogeneous cell associations arranged into metazoa – the now most simple metazoa (sponges, algae) having ≥ 3 different kinds of cells – this process probably repeated. Possibly the large differences among essentiality patterns of the said large groups of metazoans, in conjunction with the very similar ages of the least common ancestor (Feng et al. 1997) suggests that

1. Each of these groups of metazoans originated independently from monocellular organisms in
2. Multiple processes (different bacteria and monocellular algae still form temporary associations) linking organisms whose unlike essentiality patterns were due to modes of metabolism already different

The formalism which was developed to predict complex stabilities can be used to estimate extraction patterns from some environmental source also if corresponding electrochemical ligand parameters and hapticities (or/and their respective changes) are known giving information on this feature (or secondary effect) of chemical evolution in quantitative terms. Schuster (1984) showed that general autocatalytic processes in open systems – including living beings which all are open through-flux systems – give rise to the following features:

1. Selection of certain reaction modes (viz. (some of) the autocatalytic ones) and
2. Darwinian evolution becomes possible provided there is some way of storing information on the chemical structure.

Having three new functions established at once – which may be partly beyond the scope of the biochemistries of the organism's predecessors – would be highly improbable except for elements with very specific chemical (catalyst) properties at first glance. However, starting with, e.g. one single mutation in metal transport proteins, disregarding those few nonmetals which are present in most diverse chemical environments in every biorelevant organic substance, meeting literally hundreds of functions, including those of redox catalysts (N in flavines, pyridoxamin). Restricting our focus to essential or probably essential **metals** (not C, N, S, Se, As), these include V, Mo, W, Mn, Fe, Co, Ni, Cu. We thus need a realistic estimate of the likelyhoods of such processes, which must have occurred during biological evolution for a two-digit number of times; this realistic estimate takes account of at least five different factors:

1. Chirality of most biological substrates.
2. In redox-active elements like those listed above, parameters c and x depend on the ambient redox potential including changes of ambient (geobiochemical) conditions, causing "moves" in or around the "window of essentiality", unlike with, say, Mg, Ca, Zn or Cd.
3. Mutations often happen by simple duplication of some part of genome during replication.
4. Many enzymes are not exactly substrate-specific, e.g. peptidases acting as hydrolases, esterases etc. to some extent (Vallee and Williams 1968) or vice versa.
5. Endosymbiosis and horizontal gene transfer.

The first property of substrates listed here, i.e. chirality, probably was the key to their getting any functions: while prebiotic reactions afforded racemic chiral compounds, chiral "seeds" were introduced from outer space (Cronin and Pizzarello 1997; Pizzarello et al. 2008), to combine with metal complexes containing chelating ligands already then acting as protoenzymes. Often, such chelate complexes are chiral without containing any chiral ligands, simply due to the configuration in which several (and be they identical) ligands are bound to the metal center (Jordan 1994; Riedel 2004). With enantioselectivity of transformation by such catalysts being directly induced, two functions (i.e. transformation of either part of the racemic mixture) are caused straightforwardly by stochastic combination with racemic chiral compounds. For example, with sugars, such enantioselective reactions promoted by either enantiomer of a metal complex will not necessarily provide enantiomers of products, either. It is likely that chirality (in both senses of enantioselectivity of transformations and of chiral compounds existing in non-racemic forms from scratch)

was (one) starting-point of establishing functions during onset of multiple metal catalysis coupled to biogenesis: whereas

(a) "Colloquial" prebiotic transformations afford racemates as a rule, meteorites provide enantiomeric excesses as a kind of "seed" (Cronin and Pizzarello 1997; Pizzarello et al. 2008) with both (2-methyl-) amino acids and ketones capable of transferring their chiral bias to other Earth-borne molecules, e.g. during polymer formation.
(b) Obviously metal complexes acted as pertinent catalysts and/or templates from step 2 of this model to biopoiesis and beyond, binding chelate ligands like amino acids from step 2. Such chelate complexes are chiral also even if containing racemic or optically inactive ligands. Here, chirality is a result of configuration of (usually octahedral) complexes with bridging ligands (Jordan 1994; Riedel 2004).

Thus, two functions at once result from simple statistical combination of racemic chiral compounds, the adducts of (potentially) optically active substrate and (likewise racemic) chiral chelate complex forming a diastereomeric system with selective activation of one enantiomer each time. This each selective activation of one enantiomer provides products which need not be enantiomers of each other (sugars!). If there are two or more chiral centers in the substrate molecule, a metal ion catalyst gets three or more functions simultaneously if it is embedded into an environment (ligand sphere) of chiral topology or containing chiral species which thus is capable of chiral induction/enantioselectivity. This will happen in any complex which contains two bridging (chelator) ligands one of which forms diastereomers (threonine/allothreonine or isoleucine/alloisoleucine or using some mixture of sugars obtained in HCHO/CH_2(OH)-CHO oligomerization (glucose/fructose/mannose)); all these compounds can be produced in prebiotic reactions (Evard and Schrodetzki 1976). Effects of absolute asymmetric synthesis, e.g. using selective photolysis by circularly polarized light, in such systems cannot be determined yet as there are no corresponding experiments but, given the general setting, there is no need to invoke chiral photochemistry for introducing functional multiplicity (rather the contrary). Moreover, irradiation into dd bands would cause interconversion of stereo (*cis/trans*)isomers in metal complexes (Hennig and Rehorek 1987), removing the basis for separation of functions among diastereomeric substrates (sugars, tartaric or hydroxiaspartic acids, threonine etc.) and thus would be obstructive for fulfilling the three-functions-rule from scratch.

If stereochemical configurations of achiral chelators bound to metal ions are sufficiently stable, like with Cr(III) or Co(III), there is no need for enantiomerically pure ligands. Once there is enantioselective transformation of each of the diastereomers by isomeric complexes of the mentioned ion, the three-functions-rule will be stably fulfilled. If the three-functions-rule is to be fulfilled employing some (complex-bound) metal ion which processes a mixture of diastereomers by a mixture of complexes in a selective manner, configuration stability of (octahedral or square-planar) complexes is a distinct advantage. Corresponding reactions might be ammonolysis of sugar mixtures which gives various amino acids. Central ions like Cu(II), Ni(II), Cr(III) or Co(III) are stereochemically far more rigid than complexes/protoenzymes containing Mg, Mn, Fe or Zn; nowadays the stereochemical information in metalloenzyme transformations is obtained from the apoprotein entirely, with often highly symmetric, e.g. His_3Cys ligand spheres around the metal ion.

This author is unaware of any octahedral metal environment in contemporary enzymes which is chiral at the metal center itself, be it by either binding six different (all sites different, defined stereochemistry) out of 11 available ligands: this kind of chiral assembly could be constructed readily given the 21 or 22 different proteinogenic amino acid side chains present an array of **eleven** different donor sites to a metal ion (OH, phenolic OH/phenolate, NH_2, -$CONH_2$, carbamidine, imidazol, indole, carboxylate, thiolate, selenolate, dialkyl sulfide). This argument even neglects metal ion/arene ring π interactions or O phosphorylation, or various cofactor ligands, let alone the various manners of chirogenic chelate bridging. What does this tell us? First of all, the almost perfect enantiospeficity of contemporary enzymes means there is no need (anymore) for this way of producing enantioselectivity, accordingly less stereorigid metal centers, like Mg, Mn, Fe or Zn, can also be used **now** (with Mg or Zn, coordination usually is tetrahedral, thus four different ligands will do, probably including simple ones like oxamidate whilst one will be either water or the substrate).

Most likely the "blueprint" for stereoselective transformations fulfilling the three-functions-rule was one of the former rigid ions linked to a chiral environment and causing transformations in a substrate mixture

without permanent linking to the metal ion's environment, e.g. with the stereorigid and chiral – say Cu(II) – complex catalyzing an oxidation by some ambient species (e.g. nitrite) or addition of nucleophiles while being only transiently bound to some prebiotic solid. Due to then ambient redox potentials, the presence of larger amounts of Cu(II) or Co(III) is doubtful, but Ni(II) or Cr(III) might do, even allowing for later replacement by ion centers catalytically more active for the given transformations once apoproteins became stereochemically rather homogeneous (L amino acids becoming highly dominant). As Figs. 3.1 and 3.2 reveal, Ni^{2+} and Cr^{3+} are located at or way off, respectively, the right side margins of the window of essentiality, as is Cu^{2+} and perhaps also Co(III). This does suggest that biocatalytic features in a metal system bringing about chirality at the metal center would be possibly far from optimal, with a chemical-catalytic bonus to be gained by either metal exchange (returning to the "window of essentiality" altogether) or at last constructing some (much) more homogeneous ligand sphere like the above His_3Cys (many copper, zinc proteins) or porphyrin/His/Cys (cytochromes) environments (it is remarkable that the entatic effect still exists in such metalloproteins) later in evolution. Thus, Cr and Ni probably lost many functions, with chromium even getting unable to meet the three-functions-rule any longer in many (most) organisms. It is conspicuous that **metal center(ed) chirality is avoided** in biochemistry much like organometallic transformations are with few exceptions.

As an alternative to chiral "seeding" from outer space and a direct pathway into fulfilling the three-functions-rule in a kind of possible early biocatalyst, chiroselective radiolysis of amino acid (i.e. serin(ate)) complexes of various 3d-ions (Co(II), Ni, Cu(II)) and of Pd(II) (rather than just of amino acids) was recently demonstrated (Gusev et al. 2008), using both (^{90}Sr + ^{90}Y) β^- sources (helically polarized electrons) and synchrotron radiation. Most amino acid complexes of amino acids bearing additional functional groups in the side-chain yet just bind by the 2-amino- and the terminal carboxylate groups in a bidentate fashion (Kiss et al. 1991; Sövago et al. 1993). By omitting coordination of metal ions along the side chain groups complex formation constants arise which are very closely similar to those of the homologous glycinatocomplexes (Kiss et al. 1991; Irving and Williams 1953). Accordingly, the functional groups of the side chain will remain accessible for linking to other metal ions. In addition, **photolysis** of malonatoligands in (at least somewhat oxidizing and thus LMCT-activatible (Horvath and Stevenson 1992)) complexes, e.g. such of trivalent Mn, Fe, Co, Eu or Cu(II) produce carboxymethyl radical anions (acetate minus 1 H; C-centered radical) which can then add to more simple amino acids producing, e.g. aspartate – once again with a "pendant arm" – from ligated glycinate (Ferrari and Cultrera 1961). The procedure will repeat upon bridging if metal ion concentrations are sufficiently high (e.g. at metal oxide interfaces or in insoluble salts) ensuring formation of more complex biomonomers. The principal conditions for this are

1. A combination of glycinate or similar amino acids and of malonate be stable in the same coordination sphere which implies x_{2d} close to zero for the very different $E_L(L)$ values of either ligand at reasonable c values
2. Allowing then for chiral radiolysis or photolysis after linking both parts
3. Sufficiently short distances among active metal ions

There are changes in c and x values when redox potentials are changed, be it on a global scale ("Precambrian oxygen catastrophe", photooxidation of a primary atmosphere) or spatially restricted (regions which now become anaerobic (wet soil, deeper layers of stratified lakes)). From this changes new possibilities arise to bind new/other substrates in an increased number or/and to activate them at some oxidized or extremely reduced metal site, possibly even new ones (tungsten in sites of very low redox potential). In facultative anaerobic organisms (e.g. baker's yeast, *Saccharomyces cerevisiae*) some time passes by (several cell divisions) until the cells "recognize" a corresponding change of conditions and respond to it by decreasing levels of elements no longer needed (Mo) and increase those of elements now in higher demand (Zn; Moritz and Fränzle 2008, unpublished). Endosymbiosis which probably came last among the five factors mentioned above, probably gave rise first to eucaryota in general and then to the higher plants considered here, with chloroplasts derived from cyanobacteria which could until then and can still exist autonomously. Once endosymbiosis is established, essentialities of both partners "add up", as do the individual functions of a given element. Of course, each essential element in either of the precursor organisms already had to fulfill the three-functions-rule, thus the numbers of functions will increase further unless the biochemical functions are sufficiently general as to

exist in both (former) organisms in identical ways and catalyst kinds: with the 12 or 13 universally essential elements, there will be ≥ 6 functions/element thereafter.

Besides cases of biochemical redundancy, to be resolved by allocating some functions to certain organs or organelles of the metastructure, these now more than three functions can be reduced again in the long term, e.g. most Mn-containing enzymes in eucaryota are located in mitochondria while other parts of the cell often bear less than three Mn-catalyzed functions. This renders endosymbiotic cooperation essentially irreversible after it was once established and isolated cells have to find appropriate partners (it is feasible to disperse both higher plants and certain animals (sponges) to single cells and of course then to mix cell lines from different species. However, such mixtures will not form chimaeras (which can rather be made by blending subcellular entities of two species as a rule but unmix spontaneously to reconstitute specimens of either involved species in "pure" form), the only way to escape such deficits.

On the other hand, as endosymbiosis did increase the number of functions attributed to a single element, one or another metal ion in an enzyme may be replaced by some other now, e.g. Zn by Co or Cd while still respecting the three-functions-rule. This is feasible although endosymbiosis is a kind of cooperation which produces "shortcuts" in internal cooperation which by themselves reduce autocatalytic orders (Clarke 1980). Since many enzymes are oligo- rather than monofunctional (the most important being probably rubisco, capable of both carboxylating (adding free CO_2 to, then splitting the co-substrate molecule) and oxidizing (although Mg is redox-inert) ribulose-1,5-bisphosphate), SNA shows that it is a meaningful "strategy" to stabilize some pair or multiplet of enzymes and (more) multiple functions by metal exchange in or among various enzymes if their substrate selectivities are low to limited, only, e.g. in phosphatases. Vice versa, reduplication of some part of a genome which occurs rather frequently produces two firstly identical sequences which can "accommodate" two different (kinds of) metal ions and thus attend different functions from then on. The originally identical sequences will separate from each other by subsequent mutations ever after, possibly as far as to exclude catalyzing the reaction promoted by the other "copy/branch" of the enzyme any longer even if no different metal ions got involved and selectivities according to 4 are limited (cp. Yoshikuni et al. 2006). This process will occur faster if the chemical conditions in the surroundings do change. This will be discussed in more detail now.

Now let such a chiral complex, which is assumed to be chiral either by configuration around M or by inclusion of chelating ligands as modest as cystein(at)e (Neville and Gorin 1956a, b), selenocystein(at)e or aspartate in an appropriate topology, interact with some substrate containing **two** stereogenic centers, e.g. a sugar or hydroxiproline. Provided the complex induces stereoselective reactions, the specific interactions of either form with the substrate enantiomers or diastereomers will provide three different functions, e.g. in a mixture of diastereomeric compounds available from prebiotic reactions. Corresponding examples include threonine/allothreonine or isoleucine/alloisoleucine or sugar mixtures from the formose reactions, with a diastereomeric "seed" in non-racemic form obtainable from meteorites, namely, 2-amino-3-hydroxiisopentanoate and 3-aminoisobutyrate (Cronin and Pizzarello 1997). With certain metal ions (e.g. Cr(III), Co(III) (Jordan 1994), trivalent PGM ions, Pt(IV)), stereochemical configurations of achiral chelating ligands around some octahedral metal center are stable at and often far beyond RT; hence not a single enantiomerically pure ligand is required to get a chiral complex. "Modified" species such as hydrolytically fairly stable organometal entities would be conceivable also, as they do thoroughly differ from the simple aquaions with respect to c and x (see values for organotin and organolead ions in Table 2.3) but will not occur in biology except for organocobalt compounds and few alkyl- and acyl nickel species with macrocyclic coligands (porphyrines, tripeptides). Thus possibilities to "force" some metal ions which form but weakly polar M-C bonds (stable towards hydrolysis at physiological pH) when undergoing **bioalkylation into the window of essentiality are not at all exploited by living matter.** This is remarkable because the number and diversity of elements susceptible to biomethylation and other bioalkylations is fairly large, including, e.g. non-metals Ge, P, As, Sb, Se, Te, halogenes as well as metals Tl, Sn, Pb, probably Bi, in addition Cd, Hg (recall the Minamata disaster), Co, Ni, Rh, Au, Pt (Thayer 1995; Pongratz and Heumann 1999, Schedlbauer and Heumann 1999, 2000). Hg(II), Pt(II), Ag(I) and Cu(I) also form fairly stable olefin complexes, the last being relevant to cell signalling in plants (retention and signal acquisition of ethylene hormone after its formation from 1-amino-cyclopropanecarboxylic acid (Habermehl et al. 2003)).

Enantiomeric complexes thus obtained with ligands as simple as oxalate will process independently and separately (to some extent) different enantiomers or diastereomers of some substrate. The fractionated transformation of single enantiomers or diastereomers then and thus establishes and stabilizes the three functions. Hence, configuration stability of metal-ion containing protoenzymes at central M^{n+} originally was the key condition for fulfilling the rule of three functions, providing another criterion besides appropriate c- and x-values for the right metals to support or induce "first-time" biocatalysis. Unlike with Mg-, Mn- or Zn-complexes, separation of *cis-/trans-* or *mer-/fac* isomers is readily achieved with central ions such as Co(III), Cr(III), Cu(II) or Ni(II), and might also occur upon adsorption by clay minerals or carbonates. Isomeric complexes thus forming (i.e., *cis-/trans-* or *mer-/fac*-isomers as well as linkage isomers) can be separated at all oxide, silica (Fränzle 1992) and cellulose-based ion-exchanger chromatographic interfaces, hence display unlike extents of adsorption. The former metals (Mg, Mn, Zn, and others) which are not capable of forming configuration-stable complexes possibly only later got into a position to fulfil biocatalytic functions hitherto accomplished by those forming more "sturdy" complexes. For example, as is well-known (Le Son et al. 1998), in vitro peptide ligation is best promoted by Cu(II) (fairly rigid stereochemically but hard to maintain in a not too oxidizing atmos- and hydrosphere), including some autocatalytic effects due to dipeptide product ligands (ibid.; about the only established case of autocatalysis in prebiotic chemistry reported so far). Now Zn does prevail in peptide ligases (Frausto Da Silva and Williams 2001) although being less active (but also less aggressive towards biological materials if not tightly controlled than Cu^{2+}), hence – if there was any Cu(II) at early Earth redox conditions at all – the apparent optimum (cp. LeSon et al. for formation of peptide linkages) was either never "tried" or the lack of stereochemical rigidity could be accepted only after protoenzymes contained some chiral information of their own in the ligands (=precursors to apoproteins). Even though there were corresponding substitutions throughout evolution, some very narrow (except for Cu, Ca) field of parameter combinations in the c/x plot was never left in biocatalysis (cp. Figs. 3.1 and 3.2, noting that the part of Fig. 3.1 shown here is not the complete diagram but just depicting the surroundings of the "window of essentiality"; for example, group 4 transition metals Ti(III), Zr(IV) and Hf(IV) are located far below of the part shown in Fig. 3.1 for bidentate activities. This is to say that limiting conditions restricting the kinds and numbers of possible biocatalysts are derived from metal binding properties rather than ligand-effects, apart of course those which directly influence binding via corresponding values of $E_L(L)$.

4.4 Biogeochemical Fractionation Processes and Essentiality Patterns in Different Taxa Under Changing Biogeochemical Boundary Conditions

In the time soon after biogenesis, both Fe(III) and sulfate were already available as they are present in banded iron formations (BIFs; Braterman et al. 1983) and sulfate deposits exist in sediments which are ≥ 3 bio. years old. Presumably, organisms started to use these oxidants to modify or degrade organic compounds taken from their environments soon afterwards, enabling organisms to reduce biomass C/N ratios down to about 25. After dioxygen became available (O_2 being a menace originally rather than providing a metabolic opportunity for oxidative energy gains or C, N functionalizations) 2.3 bio. years b.p., biological C/N ratios, according to the empiric Eq. 2.14 could be reduced once again from several dozen at least down to <10. Although then presumably all organisms were still fully aquatic (also considering the argument by Watanabe et al. 2004), this implies much more binding sites to become open to metals from the (dissolved in the (aquatic)) environment. For fungi and what was to become arthropods (trilobites, eurypterids etc.) thereafter, this more oxidizing environment also enabled formation of massive outer hulls (carapaces) with C/N as low as 8 only (i.e., made of chitin). As another result, now metals which bind to biomass more feebly could be incorporated, binding to reduced N (amino-, carboxamide groups, imidazols, carbamidines) and S sites and possibly then become useful for (catalyzing) biochemical transformations. After the last common ancestors of protists and of all animals, higher green plants and fungi ("Luca") separated in between 1.55 and 1.25 bio. years ago (molecular dating; Feng et al. 1997), there were many additional changes in the respective patterns/arrays of essential

elements. Generally speaking, more different metals now have a biocatalytic role in those four groups of (mostly metazoan) organisms than in Archaea and other obligate anaerobic creatures, some of these forming rather weak complexes (Ca, Sr, Mn, V) or depending on sequestration by ligands the biosynthesis of which calls for dioxygen or nitrate (Mo, possibly others).

Assuming for the moment a N_2-dominated atmosphere which contains some methane, principal products of appropriate kinds of energy input will include ethyne C_2H_2, HCN, HC_2CN and cyanogen C_2N_2 (Eschenmoser 1991; Lemmon 1970), all four corresponding to admixtures of the contemporary atmosphere of large (r = 2,575 km) Saturnian satellite Titan which is composed like this, with levels of the above products except of cyanogen being limited by condensation rather than by secondary chemical reactions (White 1991) even though very large cluster anions form in the atmosphere binding lots of HCN, inter alia.

Provided Eq. 2.21 does not give a "random" effect (which would be highly unlikely) it is straightforward to assume similar effects (shifts of C/N ranges) inflicted upon the biota while adapting to crucial geochemical changes during chemical and biological evolution, e.g. in biogenesis itself, in the "Precambrian dioxygen disaster" (some 2.3 bio. years ago) or as a possible cause of rapid radiation of animals in the late Ediacarian and early Cambrian. N inclusion into hydrocarbons (CH_4 or larger) requires using high-energy ion beams (Kobayashi et al. 1989) or shock waves (denoted by)) [symbol for action of shock waves or ultrasound on chemical systems, so-called sonochemistry]; with N_2^+ or methylidine CH as the reactive precursors formed, both $N_2^+ + CH_4$ and $CH + N_2$; $N + CH_4$ finally produce HCN via

$$CH_4)))) \rightarrow CH + H_2 + H \tag{4.9}$$

$$CH + N_2 \rightarrow HCN + N \tag{4.10}$$

$$N + CH_4 \rightarrow HCN + H_2 + H \tag{4.11}$$

Or, mainly in the upper atmosphere, e.g. of contemporary Titan (Wayne 1991), via initial cosmic-ray N_2 ionization (with $N_2 >> CH_4$ (≈25:1)) by

$$N^{2+} + CH_4 \rightarrow HCNH^+ + H_2 + N \tag{4.12}$$

and recombination, then $N + CH_4 \rightarrow HCN$ …

Otherwise, it is very difficult to introduce nitrogen into nitriles or any other "prebiotic" compounds when only N_2 is present as a N-containing precursor (Ferris and Chen 1975). Apart from N_2 reduction in Fe/Mo/thiolate systems (Schrauzer 1975), it is thus likely that the first organisms which presumably formed under an N_2-dominated atmosphere contained **little more than traces of nitrogen**, i.e., the ratio C/N was very large, possibly >100, with correspondingly small shares of amino acids in the original biological matter (Mg or Ca salts of long-chain carboxylates are as poorly soluble as those of amino acids). Fatty-acid conjugates of amino acids thus formed behave as surface-active substances, with bubbles capable of proto-reproduction, offering one hydrophobic, strongly adsorbing end forming vesicles and another still (by carboxamide + carboxylate, average $E_L(L)$ about –0.1 V) complexating metal ions and concentrating them in any small cavity thus forming. Besides, very weakly acidic reactants like HCN will also enrich in such a protomembrane, ready for subsequent reactions.

Accordingly, amino acid side chains would have had a marginal role only in "primeval" metal supply of early organisms, with carboxylates prevailing which causes a certain selectivity together with the composition of seawater (Fränzle 2008a). Given the – as yet phenomenological and somewhat enigmatic – relationship between log C/N and ambient redox potential (given by Eq. 2.21),

$$\log (C/N) = -1.898 * E_{pH=7} + 1.827 \tag{2.21}$$

which determines biochemical criteria (composition of an organism) for effecting biooxidations using some oxidant in metabolism, the share of amino acids (C/N between 1.5 (arginine) and 9 (tyrosine, phenylalanine) in proteinogenic amino acids) must (have) be(en) even lower before Fe(III) became employed as an oxidant.

As pointed out before, these compounds will react among each other and also with water vapour at terrestrial rather than Titanian T levels, affording a variety of products which in turn depend on the applied catalyst. When the eventual product can extract the catalytic metal ions for this very reaction from some reservoir (solid or dissolved), the system (a) gets autocatalytic and (b) the corresponding product is selected against the others formation of which is promoted by catalysts which cannot or hardly be sequestered by their own products. So the relationship between formation and

autocatalysis must be considered to some semi-quantitative detail. Reactions between ethyne and HCN (+H_2O) afford, inter alia, pyridine, glyco- and acrylonitriles, according to

$$C_2H_2 + HCN\ \{a\} \rightarrow C_6H_6, \text{pyridine} \quad (4.13)$$

$$\{b, aq\} \rightarrow CH_3\text{-}CH(OH)CN \quad (4.14)$$

$$\{c\} \rightarrow CH_2{=}CHCN \quad (4.15)$$

whereas ethyne + cyanogene produce pyrazoline, N-cyanopyridine, oxamide, or amino acids like glycine, threonin according to

$$C_2H_2 + C_2N_2\ \{a\} \rightarrow 1,4\text{-}C_4H_4N_2 \quad (4.16)$$

$\{Cu^{2+}\} \rightarrow$ glycine (from cyanogen + H^+; Ling et al. 1977); threonine (using a combination of substrates) (4.17)

$$\{Co^{2+}\} \rightarrow H_2NCO\text{-}CONH_2 \text{ etc} \quad (4.18)$$

and

$NH_2CH_2COOH\ \{Mn^{2+}, Al_2O_3; \Delta\} \rightarrow$ various products (Ivanov & Slavcheva 1984) (4.19)

respectively, when {a}, {b} and so on are metal ion catalysts which remain yet to be identified. {a} is likely to be Mo (Berthelot-type cyclotrimerizations), b can be Co^{2+}, Hg^{2+} etc., while the Reppe transformations (Ochiai 1968; Riedel 2004) suggest c = Ni. Now for the second part of the argument: carboxylic or even amino acids perform poorly in extracting Mn(II) or Al from some substrate, with a = Mo autocatalysis becomes moderate at best. However, if there is cyanogen, the mobilizations of both Cu and Co by the corresponding products will be strong, given the stabilities of Cu amino acid (Irving and Williams 1953; Sigel and McCormick 1970; Le Son et al. 1998; Kiss et al. 1991) and cobalt carboxamide (Cotton and Wilkinson 1981; Sigel and Kapinos 2000) complexes, combined with some reactivity to cleave them again. So the present essentiality of either metal – although not universal (Emsley 2001) for cobalt – may be taken for an argument that cyanogen C_2N_2 was available on the early Earth, with corresponding drawbacks to sugar phosphorylation and other condensation reactions (Degani and Halmann 1971; Rauchfuß 2005). Cyanogen is a nitrile (i.e. oxalodinitrile), hence $E_L(L)$ ($\approx$ 0.35 V) falls well short of that of CO, avoiding the above problems. Even its alkaline hydrolysis would provide products useful in prebiotic chemistry, namely CN^- and NCO^- (condensation agent, precursor to cytosine, to amino acids (by reactions of 2-hydroxiacids with cyanate)). So it comes to no surprise that mixtures containing these elements (Sakurai and Yanagawa 1984; Kobayashi and Ponnamperuma 1985b) afford appreciably more amino acids than are obtained without them. Autocatalysis, by tapping mineral resources, would even enhance this effect and give an advantage in reproduction to entities (whether these were already "alive" or not) which contain and "use" them. Cyanogen probably was one key compound, thus.

Distribution patterns in the eobiont or "last common ancestor" (or even still in "Luca") will be unlike those in other structures of the same time and site(s), hence metal abundances correlating good or worse or negatively with those in the protobiont. Conceivably, selection/positive fractionation of essential metals in the protobiont and giving them the required three functions would distinguish this from all the "inactive", at least, non-reproducing varieties. This is not to say that there already was non-equilibrium fractionation by some kind of, perhaps photochemically induced, active transport (like in banded-iron formations), but one result of this which corresponds with recent-time BSE findings will be likely: very low to non-existent abundance correlation between essential and non-essential yet chemically similar elements of the same PSE group, like it was observed (Markert 1996) in, e.g. the couples P/As or Ca/Ba. This is observed although the "Biological System of Elements" data came from comparisons among different contemporary, somewhat more closely related living beings, not between the former and, say, the metal affinity patterns of some marigranule or microsphere.

Likely products of step 2, precipitates of Zn or Ca salts of amino acids valine and isovaline, were subjected to heating to 320°C in a nitrogen atmosphere (Strasdeit et al. 2001), causing complete thermolysis. Besides the simple diketopiperazines (cyclic dipeptides) and other peptides, heterocyclic compounds arise, and ketones (namely, the symmetrical Ruzička products which form from Ca salts after desamination of the amino acids, like 2,8-dimethylnonan-5-on) and other "prebiotically feasible" small molecules. As might be anticipated, product yields and distribution sensitively depend on whether Zn or Ca ions were involved. There is some quite simple precondition for

steps 1 to 3 taking place consecutively by and with metal ion loading of the prebiotic polymers or precipitation products: in step 1 average complex stabilities must increase, thereafter giving rise to metal loading and catalytic activation.

Except for La(III), critical (minimal) electrochemical ligand parameters are so low for all the rather abundant metal ions as to provide some substantial gain in binding energies and complex stabilities during chemical evolution, causing step 3 to occur spontaneously: there are no (plausible) anionic ligands with $E_L(L) > +0.40$ V. Although abundances are sufficiently high for Y, La – Nd, Sm, this kept REE ions from becoming part of primeval biomasses, regardless of their recent biological activities: support of cell budding in various species, activation of phosphatases up to the point that these metals are efficiently precipitated from their citrato complexes as phosphates, unlike with thorium or Pu(IV) (Yong and Macaskie 1997). Data in column 6 (Table 4.1) show in addition, that metal ions get attracted from some solution exactly when log $k_{chelate}$ is larger than the value given here, implying minimum concentrations of metal ions which must be present in "primeval soup" or in contact with some corroding or eroding solid material supporting the products of steps 1–3. Now, chemical evolution gives rise to chelating ligands which consistently have larger electrochemical ligand parameters than the minima (that is, > some −0.30 V), yet the latter does not hold for all metals: the mechanism of enrichment is selective anyway, except when phosphorylated species are formed, like with the photopromoted system cyanide/phosphate/Fe(III) (Saygin 1984) which produces carbamoyl phosphate. Corresponding organophosphates (esters etc.) are distinguished by substantially lower $E_L(L)$ (e.g., −0.37 V {calc.} for glycerate-3-phosphate) and, in addition, act as PO_4 transfer agents which in turn convert other ligands which, like sugars, serine oder hydroxicarboxylic acids have (sufficiently) higher $E_L(L)$, into compounds which resemble the original organophosphates with respect to metal ion selectivities.

As a result, any excess of phosphate in conditions enabling, e.g. photochemical phosphorylation would end up with organic materials rich in phosphate attracting Al^{3+}, REE ions on expense of its own stability towards hydrolysis (as REE^{3+} promote organophosphate degradation). This is to say that step 3 would take place if and only if intermediates can discriminate against REE ion take-up (and precipitation of inorganic Al, Fe, Mg), exactly enabling a later-on function for controlled signalling – competing with Ca^{2+} for the latter "task". Phosphate-rich materials would thus be excluded from further chemical evolution even though, e.g. corresponding carbohydrates would form readily (Eschenmoser 1991). We shall soon see that, judging from $E_L(L)$ and denticity changes during chemical evolution, this is a real(-istic) property of the putative primeval polymers. Apart from this case, complexation equilibria would efficiently discriminate now generally essential elements – taken up from solution – vs others, with the latter including Al, Cd, Co(II), Cr(III) and UO_2^{2+}. Metal ions with corresponding properties could, if at all (Co, Cd, Cr), attend biocatalytical functions and thus essentiality only much later whereas they simply remained in solution (or locked up to mineral supports, with no way of leaching) in the early days. Thus it can be taken for sure, that

1. Primeval biomass could not retain REE ions.
2. There is but a very narrow region of pH values in which the nowadays essential elements can remain dissolved in such amounts that they (all) were prone to parallel extraction to and by some solid polyligand (step 2 of the above model): although, for example, recent seawater contains sufficient Mg^{2+}, but the concentrations of Cu, Fe or Zn are way too low for its alkalinity (pH 8.3), whereas recent freshwater does contain adequate amounts of Mo or Fe but too less of the other (essential) elements to have them bound by precipitates of insoluble salts containing simple ligands.

However, there is another scenario which can escape this problem: (perhaps photoassisted) synthesis of amino- or oligocarboxylic acids on some mineral interface, to be followed by chemical corrosion of that interface much as modern lichens or cryptoendolithic organisms do. When, in later phases of chemical evolution, chelate ligands do already prevail and can be interconverted (and even more activated), e.g. by photochemical processes, the set of lines from Fig. 2.15 applies analogously. This is shown in Fig. 4.1. Figure 4.1 also shows that, although $E_L(L)$ of the chelators use to be considerably smaller than in some pertinent monodentate ligands such as NO_3^-, NO_2^-, oximes or CN^-, chelate complexes forming in steps 2 and 3 are far more stable owing to higher c and often positive $x_{>1d}$ values of the metal ions. The chelate effect will prevail if no neutral monodentate ligands with very high $E_L(L)$

like CO or alkenes can enrich intermediates; this criterion excludes possible palaeoatmospheres with large amounts of either CO (i.e., very dry ones, see above) or CH_4, alkenes C_2H_4 or l-C_3H_6 (and the similarly behaving alkynes C_2H_2, C_3H_4) and polyynes like C_6H_2 then being intermediates in photochemical smog formation in N_2/CH_4 atmospheres (Strobel 1982; Lewis and Prinn 1984; Wayne 1991).

The fact that Mg, Fe, Zn and Cu, Ca are the most prominent metal ions in biology – also in quantitative concentrations – thus can be traced back to selective enrichment already during chemical evolution, i.e. prior to any "function" which could have been directly related to reproduction of whatever entity. This early system would **not reproduce chance distributions** of metal ions at certain "special" sites, e.g. mineral springs; accordingly, as the recent patterns of essentiality are linked to selective extraction (even though it now requires some lability, that is, binding of metal ions in and to metalloproteins "must not" be too stable) the information to be gained on sites/hydrochemical conditions of biopoiesis is limited. Yet, even assuming moderately reducing conditions, the pattern of extraction would rather be linked to the ion composition of (however recent freshwater (Markert 1994b), interacting with an oxidizing atmosphere than to that of the ocean (Nozaki 1997), the latter being fairly stable over long periods of time (Sillen 1964). Of course, the same pattern of selective take-up would arise if

(a) Metal ions from solution interact with a solid phase which contains the respective ions in similar amounts (excess levels of Mg, Ca compensated by their forming weaker complexes than 3d ions (see Irving and Williams 1953; Sigel and McCormick 1970)) or
(b) Some (mineral) solid phase would retain the ions to comparable extents.

Carbonates are one plausible solid source for metal ions which display similar dissolution/complexation barriers for various ions (that is, different CO_3^{2-} salts, e.g. of Fe(II), Mg, Cu, Zn or Mn(II), are similarly soluble in water (pKs ≈11; Mizerski 1997)) and form mixed crystals among each other. In addition, the products of chemical evolution, with HCOOH, many higher carboxylic acids with or without amino groups being prominent in yields (Lemmon 1970), are usually acidic and thus would dissolve carbonates readily. Contrary to carbonates, other kinds of minerals like phosphates, silicates or sulphides differ much more with respect to aqueous solubilities and are also less susceptible to acid attack. Hence, if

(a) The recent essentiality patterns correspond to selective extraction from some solid phase rather than from water (of whatever hydrochemical origins and salt contents) around the times of biopoiesis and
(b) Are not completely due to chemical properties being somewhat similar as suggested by the "essentiality maps", then

carbonates are the most likely precursors, and should be considered abundant under a weakly oxidizing atmosphere. After both mechanical and chemical erosion of solid phases in Earth's crust, precipitation in seawater brings about some fractionation (Fig. 4.2).

Concerning minerals formed and possibly involved in this part of chemical evolution, one cannot now proceed much beyond such general reasoning because even though there is some information on metal ion retention on salt interfaces in the geology and geochemistry/mineralogy textbooks, electrochemical ligand parameters for a solid/aqueous interface cannot be inferred from those retention data unless known in solution, e.g. with fluorides, carbonates or phosphates the solubilities of which are well-known. Here, $E_L(L)$ is about −0.4 V, implying step 1 of the above scenario would occur on some mineral interface also without any contributions from the atmosphere. For example, the presumably Archaean minerals ZnS (wurtzite or zinc blende), perowskite $CaTiO_3$ or ferric oxide are active photosemiconductors which thereby undergo anodic photocorrosion generally to release trace admixtures: consider

(a) The photolability of many sulfide minerals, particularly such containing As and Sb like realgar, tennantite. and
(b) The rapid photoreductions of perowskite, releasing Ti(III), and of MoO_3, providing reduced Mo, both causing some blue to violet tan of the solid. Subsequently Mo^{red} causes reductions of dinitrogen (Bahadur et al. 1958) while Ti(III) effects coupling among carbonylic organics.

Then transformation of these intermediates in presence of carbonate, nitrite would let step 1 go on, ending up with the recent metallome, unlike with the "RNA-world" or under a primeval atmosphere containing substantial CO (Miller and Schlesinger 1984; Kasting 1993) which would break the sequence of

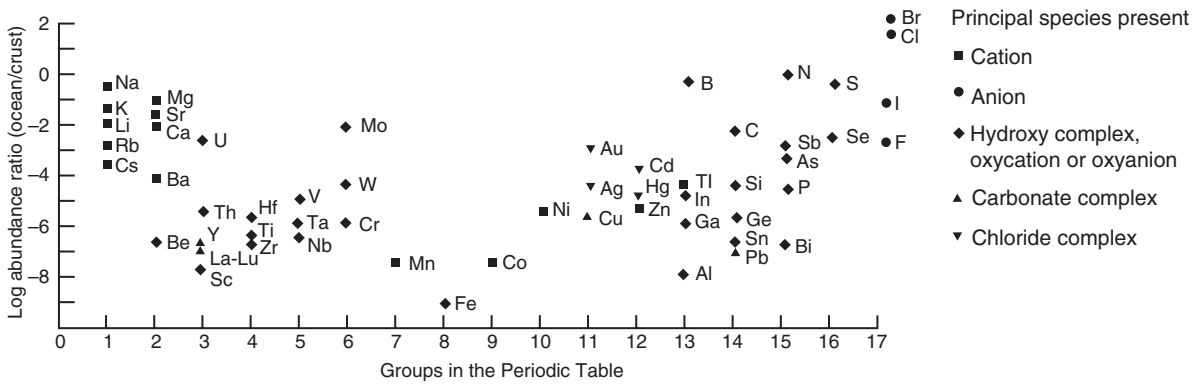

Fig. 4.2 Fractionation and speciation (*right side of diagram*) of chemical elements after erosion of Earth's crust and input into seawater (from Frausto Da Silva and Williams 2001). Except for N, Cl and Br, all elements are relatively depleted, essential ones other than K, Mg and Mo even to a very high extent. Concentrations of important metal ions which can be maintained at recent pH (8.3) in seawater are far too low to realize steps 1 through 3 in seawater. Concerning the long term, including thermodynamic, stability of now abundant chemical species in both hydrosphere and atmosphere cp. Sillen (1964, 1967), reproduced with permission

events to yield quite different patterns of metal ions in the condensed organic phases forming there. Even though there is no definitive statement whether the metal ions were taken from solution or from (probably photoactive) supports on these premises, some partial reconstruction of environmental conditions during biopoiesis is feasible, both for early Earth and other possible (former?) abodes of life, such as Mars. The contemporary Martian atmosphere is distinguished (Fegley Jr 1995) by short-time variations of both CO and O_2 (and also CH_4) abundances which means either CO_2 photolysis, or, rather recombination rates along

$$H_2O + h\nu \rightarrow H + OH \tag{4.20}$$

and

$$CO + OH \rightarrow CO_2 + H \tag{4.21}$$

$$H + O_2 + M \rightarrow HO_2 + M \tag{4.22}$$

$$HO_2 + h\nu \rightarrow O + OH \tag{4.23}$$

vary considerably within a few years of time while the regolith dust surface is not fully oxidized – although containing compounds/ions like NO_3^- and ClO_4^- – which is shown by its being ferromagnetic, indicating the presence of maghemite Fe_2O_{3-x} at least (if not even magnetite) in contact with the atmosphere (which, moreover, receives some CH_4 from unknown but regionally confined sources). The required absence of CO implies this old atmosphere to be rather moist to avoid accumulation of CO under the given intense UV radiation.

Given this, the "natural selection of the chemical elements" (Williams and Frausto Da Silva 1996) now essential for more or less many different organisms (e.g. including V, Cd, Co, Ni) extended – even leaving aside effects of combination during formation of eucaryota and later metazoans – over longer periods of time: if selective enrichment of the "basic set" of elements was feasible from carbonate supports or freshwater rather than be obtained from the early oceans, several of the above were also enriched in the going-to-be biomass but caused problems rather than got a function in most organisms for the times to come (e.g. Cd). Accordingly non-random processes with a certain extent of specific transport took over, yet, the present (usual) toxicity of Al, Cd or Cr results from later developments putting such ions into competition with others already fulfilling functions (particularly, Zn), additionally causing membrane attack by complexation (U probably was not yet hexavalent then and unlikely to undergo spontaneous enrichment in proto-biomass(-es)). This reason of heavy metal toxicity prevails in metazoans, particularly in animals, with Al and Cd sticking to gill epithelia (Paquin et al. 2003) while U(VI) and also Cd destroy kidney membranes.

The role of phosphorylated compounds requires further considerations, particularly considering the problems pointed out before in proceeding beyond step 1, given their fundamental roles in energy metabolism, activation of molecules, signalling and eventually inheritance.

The idea of "RNA world" implies proto-biochemistry being controlled by polyphosphate ligands at a period of time where there were not yet any specific metal ion-sequestration properties. A calculation of $E_L(L)$ from complexation equilibria of many different metal ions – both di- and trivalent ones – with RNA or DNA of different origins (data in Izatt et al. 1971) and Eq. 2.10 yields a value of −0.30 V; accordingly, the ligating site is the polyphosphate backbone of NAs, not the heterocyclic compounds on the "inner side" (if coiled up) or the sugars acting as spacers and links in between either. Hence, insofar as present roles of Mg, Ca and Zn ions in NA transformations can be extrapolated that far backward in time, NAs would not have been capable of doing similar ribozyme-style self-processing without using other – non-NA – agents to obtain the above ions. In addition, SNA arguments strongly suggest autocatalytic (reproducing) systems which rely on but one kind of polymer to be highly unstable at best (S. Fränzle, unpublished). So the "classical" RNA world would not have accomplished its maintenance; rather, it must be assumed that the ratios C/P and N/P in the early material should have been considerably higher than nowadays in biomass to compensate such effects.

Nowadays, activities of nucleic acids are controlled by interactions of Mg, Ca or Zn, but also by heavy-metal ions or electrophilic agents with certain specific sequences (e.g. in induction of metallothionein). Though this does imply NAs to act as ligands in physiological conditions, it need not imply that they could achieve the above sequence of steps, let alone the problem that up to now not even a hint of a prebiotic NA synthesis pathway was demonstrated, not even when using polyphosphoric acid in organic solvents. The $E_L(L)$ values for NAs, nucleoside triphosphates or simpler species such as glycerinaldehyde-2,3-diphosphate are way too low to permit the sequence of events on their own.

As the CO content of an "effective" atmosphere had to remain rather low (unlike that of other possible intermediates like cyanogen (oxalodinitrile)) to "run" chemical evolution with metal uptake continuing beyond step 1, sufficient OH had to be present in order to reconstitute CO_2. In addition, OH also promotes transformations of hydrocarbons, including formation of polymers via radicals and alkenes and functionalization of higher organics (aromatics, glycine-dominated peptides). Although mineralization of organic intermediates would be another possible result of excess OH presence, the fact has to be acknowledged that – except for CH_4, glycine and few other species – the organic compounds are **metastable, at most**, transient intermediates upon prolonged (direct or indirect) photooxidations in any case. That is, chemical evolution depends on "quenching" these organics by, e.g. precipitation much before completion of these reactions. Notwithstanding this, deuterium is considerably more abundant (156 ppm) in terrestrial water samples (SMOW) than in gas samples (CH_3D, HD, HDO) of the large Jovian planets (30–40 ppm) (Lewis and Prinn 1984). This implies that substantial hydrogen (H rather than D) losses by water photolysis occurred in the upper early atmosphere, like on Venus but less pronounced, which implies, that the stratosphere, then, unlike the present stratosphere, obviously had been moist. OH was a principal product of corresponding processes.

It needs consideration that substantial CO (or alkenes somehow escaping polymerization) would have stopped the process, notwithstanding the presence of CO ligands in hydrogenases (besides cyanide, thiolate) which (therefore?) often are considered "primitive", "old-age" enzymes. Considering hydrogenases to be "primordial" enzymes does include the assumption that there either was considerable H_2 as a substrate in the early atmosphere or steadily afforded by, e.g. the pyrrhotite/pyrite conversion or by Fe(II) photooxidation (Braterman et al. 1983; Horvath and Stevenson 1992). The former causes problems with dihydrogen retention because of Earth's not too strong gravity, whereas the latter requires an abundant source of H_2S (volcanoes?) or of Fe^{2+} (peridot weathering?). Enzymatic methane formation from CO (by Ni porphyrines) or production of acetate by clostridia in much the manner of the Monsanto process surely developed much later (*Moorella thermoacetica*, using a Cu or Ni carbonyl attached to a tetradentate ligand bound to Ni, the latter containing glycine, cysteine and another S donor).

Today, there are enzymes in which Cd or Co do replace Zn upon changes of ambient water chemistry (Price and Morel 1990), letting one to presume this might be a relic from times where distinguishing and allocating metal ions was not yet a highly developed process but protocells had to run metabolism with those metal ions which accumulated inside them almost at random – except for complexation selectivities. Rather, such replacement is found in **aerobic** organisms, concerning transformations and enzymes which were not required in early life, like with carboanhydrases.

Both Cd and Ni being clearly within the window of essentiality, thus having coordination properties "suitable" for bioinorganic transformations, their recent toxicities to many organisms must be an outcome of only later developments in evolution, possibly linked to radiation of metazoans, given the prominent role of nickel in plant (unlike animal, fungal) biochemistries, with Ni used there often instead of Mn or Zn in other creatures (e.g. with urease) and (several) plants also causing/tolerating Ni hyperaccumulation. However, it might also just correspond to narrow "therapeutic windows", much like with other ultratrace essential elements like V, Mo, Se etc. Likewise, Cd essentiality is far better established with plants (Strasdeit 2001) than with animals.

Conceivably, with other elements "cumulating" functions during "assemblies" of (1) eucaryotic cells and then (2) metazoans, these metals, although hitherto operating in accord with the three-functions-rule, were out-competed by some other elements providing similar or better catalysis for most reactions (except those involving dihydrogen or CO). In animals or their predecessors, one such metal likely was Mn, while it is hard to identify any (let alone 3) biorelevant reactions best promoted by Cd complexes. Accordingly, Cd was used up to then (if at all, with the organisms not yet requiring CAs then) just because it was ready to obtain. With the two steps increasing intracellular and organismic complexities in the second-last eon from now, Gibbs's phase rule took over to control "acceptable" complexity among essential elements in a way not seen before. Similar arguments might hold for chromium(III).

Once biogeochemical redox conditions do change, redox-active metals such as V or Fe or Ni, Cu respond to them by changes of oxidation states and thus (also) of c and x values (cp. Table 2.3). The most eminent such event was the "Precambrian oxygen catastrophe" (2.3 bio. years BP). Metals such as V, Mo, Fe and Cu, possibly also Co, which possibly existed in lower oxidation states earlier than 2.32 bio. years BP then had other c and x values, less positive x_{2d} and likewise decreased c_{2d}. Possibly the Precambrian oxygen disaster thus did influence access to Fe and Co only while the photosynthetic key metals – and those of the tricarboxylate cycle – Mg and Mn were untouched by this grave change. Neither is it feasible to distinguish those metals which could already be functionalized in association with phosphate or organic (e.g. whewellite, moolooite) anion minerals. For the anion- or thiocomplex-forming metals V, Mo and W, possibly also Cu, this popular assumption need not hold: thiometallates $MS_{4-x}O_x^{2-;3-}$ are highly stable in the highest formal oxidation states V(V) or Mo, W(VI), respectively. Their visible-light photochemistry (Horvath and Stevenson 1992) might provide an interesting alternative route to early photosynthetic reductions of much biological value but clarifying this will take additional experiments. Accordingly the very presence of sulfide would have preserved the present binding features and selectivities and the metals V, Mo and W without any need for oxygen; reduction to V^{3+}, Mo^{3+} etc. would have occurred only in rather acidic environments (pH < 4) and strict absence of H_2S. Due to formation of soluble polysulfidocuprates(II), the situation might be similar with copper. Either structural motif can be found in abundant archaean minerals.

With changes in c and x (which some organism may also organize in certain compartments of its body, given the release of highly reducing or oxidizing species from stannane up to Mn_{aq}^{3+} and elemental chlorine), other substrates may be bound to the corresponding metal centers and be turned over at some oxidized or highly reduced metal site (sulfate or phosphosulfate at Fe_xS_y centers (→ reduction; Mengel and Zickermann 2007); H_2 at Fe, Ni (either way); photoassisted water oxidation at $Mn_4^{ox}Ca$ (PS II)). Eventually, endosymbiosis is listed at last, fifth position also because it was latest in time, probably giving rise to both eucaryota and those higher plants (≠ algae, mosses, cyanobacteria) mainly considered in this book. For example, chloroplasts are considered to be derived from cyanobacteria hitherto (and still) capable of living on their own, and by their integration into animal tissues up to today (marine snails, zooxanthellae in stony corals), maintaining photosynthesis, they recall the beginnings of endosymbiosis even nowadays. When this happens, involving organisms distinguished by their biochemistries (identical ones would just form some colony without novel biochemical features, like some bacteria do or individual polyps when re-forming corals upon pH increase), will at best "add up" the numbers of functions of elements allowing for essentiality. If the same task can be accomplished by different metals, say Co instead of Zn in some enzyme, this might do to fulfil the three-functions-rule. Apart from this, "addition" of biocatalytic chemistries causes any of the elements essential to both precursor organisms (that is, the 12–13 "universal" bioelements) to get up to six functions.

Yet evolution went on, rendering cooperation and endosymbiosis irreversible for a reason given below: even if the functions were not redundant, the components of this new metaorganism did no longer need to be able to reproduce on their own. So biocatalysis of the now-organs or organelles could be optimized beyond the threshold of the three-functions-rule: in animals, most Mn-using enzymes are located in mitochondria only, with three functions of Mn occurring (also) elsewhere (malate enzyme, urea release from arginine in the urea cycle, some SODs (Cu + Mn)) only in certain aerobic organisms (many vertebrates also do not produce urea but rather release ammonia). Acidic phosphatases may contain and operate on Mn(II) also but in these enzymes Mn can be readily replaced by other metals (Mg, Fe(II), Zn) (Kaim and Schwederski 1993) and thus they cannot be considered to contribute any indispensable "function" to fulfilment of the three-functions-rule.

Once any one of the formerly independent partners did reduce or reorganize its partial biochemistry so far that the three-functions-rule is no longer fulfilled with respect to any "crucially essential" element, that is one distinguished by features that imply its use in biochemistry as we know it on Earth, the cooperation has become irreversible. On the other hand, increase of numbers of functions beyond 3 upon endosymbiosis now allows for substitutions of one metal by another in an enzyme, say of Zn by Co or Cd (*Thalassiosira* algae contain chloroplasts etc., hence are "constructed" organisms though being unicellular) and gives a blueprint for "optimization". Obviously, this need not imply an increase of the number of essential elements, rather a decrease might occur. Nevertheless, now another effect (Clarke 1980) from SNA becomes relevant: individual autocatalytic cycles are generally "weakened" to form usually critical ones by endosymbiosis-like cooperation and internal coupling producing kinds of "short-circuits" (ibid.). So cooperation is not a blueprint for stabilization although metal exchange – inevitably removing the involved enzyme from the list of contributors to the three-functions-rule – will also help for enzymes whose substrate-selectivity is limited. It is yet intriguing that the largest numbers of essential elements are required by animals, being heterotrophic, thus in no need to accomplish very difficult transformations, but anyway it represents the latest "innovation" of evolution among the various kingdoms of life (upper Precambrian).

Gene duplication is fairly abundant, allowing for production of two slightly different proteins containing the same metal ion but upon further mutations to be used in different ways. After some period of time, amino acid sequences of the two enzymes become that much different as to preclude catalyzing the function of also the other one any longer (cp. Yoshikuni et al. 2006). Once either the environment of the entire organism or its internal organization change, this process can be supported.

Ever since chemical evolution took place, presumably in some rather redox-neutral milieu, allowing to convert rather "poor" ligands (N_2, CO_2) into efficient chelators by use of appropriate catalysts (Mo for nitrogen-, Fe (+S) for carbon and sulfur reductions), chemical conditions did change thoroughly, also due to biological activities. All the systems chemically coupled to the biota did change almost simultaneously also; the pattern of stable (Sillen 1967) dissolved speciation forms or minerals of elements changed like biological material had to do (evolution of dioxygen-, nitrate reduction enzymes, SODs, peroxidases) to cope with the new challenges in other ways than by receding into anaerobic "retreats" maintaining lower redox potentials. Living beings mainly extract their essential elements from the hydrosphere or weathered lithosphere or (to a smaller extent; Fränzle and Markert 2002b) from other living creatures (heterotrophs are ubiquitous (deep ocean!) but their total biomass is much smaller than that of plants, algae). Hence these changes also influenced availabilities of redox-active bioelements, e.g. Mo and Cu (increase) while altering c and x values and thus interaction with possible substrates in metal-centered biocatalysis. So some elements – besides Cu and Mo also V, Mn, Fe, Co – "shifted around" in or next to the "window of essentiality" while positions of redox-inert bioelements such as Mg, Ca, Zn remained as they were. Many organisms are capable to maintain some internal milieu allowing an unchanged allocation (Tottey et al. 2005) of elements inside the body but to achieve this, they still had to respond to changed availabilities/chemical potentials of these elements around them. This example necessitated development of novel sequestration agents (e.g. polyphenol-functionalized peptides, polylactones, hydroxamates) delivered to soil by bacteria, green plants and fungi. Oxidation of the above metals brought about some increase of c_{2d} (between 0.5 (Sr, Ba) and 9.04 (Cu) for divalent ions; between +2 (Nd)

and about +12 (Fe, Al, Ga) for trivalent ones) concomitant with (usually) a strong decrease in x_{2d} for metals to be oxidized. The obvious strategy for metal sequestration after oxidation of the latter then is delivery of ligands with distinctly negative $E_L(L)$: hydroxicarboxylates have $E_L(L)$ between −0.13 and −0.25 V, hydroxamates about −0.20 V (Lever 1990), polyphenol(-ate)s close to −0.3 V (Rocha et al. 2002). As a corollary, biosynthesis of both hydroxamates and of polyphenols require Mo and Cu to be used in oxidized states themselves: N-hydroxy-compounds which can react with esters (e.g. lipids) to yield hydroxamates require NO biochemistry or nitrate reduction while ring-site hydroxylation of benzenoid aromatics is accomplished by Cu proteins, involving at least Cu(II) intermediates.

As shown in Table 2.1, the four large groups of eucaryota (plants, fungi, animals, protists) strongly differ with respect to essentiality patterns, these differences obviously being related to adaptive radiation of these groups some 1.3–1.55 bio. years ago (Feng et al. 1997, using a "molecular clock" derived from protein sequences). Certain groups of organisms apparently "prefer" to enrich and (biocatalytically) also use certain elements, e.g. Ni and W by archaea and clostridia, that is, organisms adapted to very low redox potentials (Ni also occurring in ureases and being hyperaccumulated by some number of green plants), V in ascidia (function as yet unknown), holothurians, fungi (Lepp et al. 1987) and also in plants producing large amounts of linolenic acid (Emsley 2001). Presumably different ways of using the above redox-sensitive elements predated the Precambrain oxygen disaster.

Figure 3.1 contains a slightly inclined line which connects many of the universally essential metals (Ca, (Mn), Fe, Mg, Zn, Cu) and others required by differently many organisms (Sr, Cd, V, Ni (Co)). Other, toxic divalent ions are more remote from this line (Be, UO_2^{2+}) while another (lightly-tanned) line holds for trivalent ions which, as a rule, lack biological function. The green line thus reveals a relationship between c and x values – some correlation between them – which has some drawbacks in (enabling or precluding) biological functions, too. Here, increasing c and x "compensate" each other if the metals are linked to some ligand of appropriate (-ly negative) $E_L(L)$ suggesting some common step or pathway in metal ion (biocatalyst)-biomass interaction, that is, the kind of ligand every metal will encounter in every living being. The respective $E_L(L)$ is −0.241 V, corresponding to $-\log k_{diss} = 4.0$. This value is in between those for phosphorylated compounds and for hydroxicarboxylates or fatty acids, suggesting links to energy metabolism and nucleic acid biochemistry, yet one should be cautious about historic conclusions.

The number of essential elements changed after the Precambrian oxygen disaster considerably, increasing to > 20 in many animals. Therefore it would be too straightforward a conclusion that metal-biomass interactions was controlled by biogenic ligands having $E_L(L) \approx -0.24$ V for all periods of biological evolution. Metals being in reduced states >2.3 bio. years ago (V, Mo, Fe, Cu, probably Co) then had different x_{2d}– and reduced c_{2d}–values (see above). Like we argued before for ligands of similar $E_L(L)$ values (the same hydroxicarboxylates, hydroxamates and polyphenol species), interactions of V, Mo, Cu or Cu with biogenic phosphates or carboxylates should have been considerably weaker then while there was no obstacle, e.g. against Mn or Mg exerting the same functions in the tricarboxylate cycle as today.

Another important issue is whether the "discrepancies" between "optimal" and "actual" ways of metal-centered (bio-)catalysis listed in Table 1.1 might likewise be due to former difficulties in binding and activating substrates in still reducing environments – if these substrates then had any importance to biology: among the metalloproteins actually containing and using V, Mo or Cu, some, like the SODs, would be of any use only in a dioxygen-containing atmosphere, while haloperoxidases (bearing V or Fe) would have been restricted to the traces of hydrogen peroxide photochemically admixed to rainwater for biochemical oxidations/hydroxylations (today the average level of H_2O_2 in rainwater is about 0.2 mg/L (6 μM)). Due to lack of an ozone layer, tropospheric UV inputs and thus H_2O_2 production should have been more prolific in those times. With metazoans not yet existing, intercellular communication by NO or ethylene (plant hormone), either undergoing complexation to Cu(I), was not significant either. Hence enzymes dealing with NO, O_2, H_2O_2, including haloperoxidases, can be omitted from consideration of Table 1.1 concerning metalloproteins which are or "should be" using V, Mo, Cu or Co, leaving to explain the following cases of apparent deviation:

- Organophosphate hydrolyses including those of NTPs and nucleic acids
- Guanidine hydrolysis (urea cycle, N recovery)
- Polycondensation of amino acids (peptide ligation)
- Oxidations of methyl groups and of CO

All but possibly the last-mentioned of these transformations were crucial much before the Precambrian oxygen disaster. In biocatalysis of all these reactions (rather) redox-inert Mn, Zn or Mg are used rather than Co or Cu. Possibly (cp. Williams and Frausto Da Silva 1996) Cu – then most likely Cu(I) – was hard to obtain whereas Mn, Mg or Zn did not respond to weakly reducing or neutral conditions. Fe(III) or Cu(II) which are quite potent and versatile redox catalysts could then not be maintained in substantial concentrations, likewise V should have been in some reduced state (for the corresponding Pourbaix diagrams, cp. Kaim and Schwederski (1993), Coleman (2003)). Molybdenum, on the other hand, could remain dissolved in various oxidation states. Presumably this is why Mo has such a prominent role in reductions of N_2 (cooperating with Fe sites) and of nitrate (which can form photochemically also in an atmosphere without free oxygen), oxidation of sulfite or disproportionation of aldehydes including aldosugars.

As pointed out before, organometal species are most uncommon in biochemistry (Williams 1986) except for cobalamine and later biomethylation products given away to the environment rather than taking part in any subsequent biochemical process (exceptions: Co, perhaps As (Irgolic 1986) which is not a metal), thus involved in detoxification. Adaptations with respect to transport (Fe) and regulation (Cu) got imminent in a now oxidizing environment.

Possibly, as presumed by Frausto Da Silva and Williams (2001), some hitherto essential elements lost their biocatalytic functions altogether or are about to do so (ibid.); yet, this issue is open to speculations only because there is no way to determine former biocatalytic functions in fossil samples even if they are fully preserved, like inclusions in amber (with chitin retaining the metals) or dry mummies. Moreover, very few such samples date back beyond the Cretaceous (dinosaur xeromummies from Mongolia, Arabic amber) whereas most of the changes we consider here took place far back in the Precambrian. Changes of environmental conditions may also contribute to stabilization of changes by substitution of some redox-inert ion with >> 3 functions by another undergoing redox processes (say, of Zn by Co in carboanhydrases and (bacterial) nitrile hydrolases or of Mg by Mn or Fe in acidic phosphatases) since the latter can be fixed in biological materials exactly due to redox fractionation. In phosphatases which are coupled to redox processes directly via proton pumps, chemical variability is large: redox-inert central ions Zn and Mg exist besides oxidizable ones in the active centers (Mn, Fe(II); Frausto Da Silva and Williams (2001)).

To give a summary, biopoiesis, the origins of life on Earth (single or multiple, omitting the concept of panspermia here) was not simply a fundamental act of emergence (Müller-Herold 1984[1]) but also likewise linked to a change in structural complexity: whereas metals (metal cations + V, Mo) and their complexes had a very limited role during chemical evolution, just increasing yields and selectivities of a few transformations (Bahadur and Ranganayaki 1955; Follmann 1985; Kobayashi and Ponnamperuma 1985b), they become crucial in the very moment of biogenesis for organizing autocatalysis in a manner required for even rather error-prone (Eigen 1971), modest-rate reproduction. It is tempting to say that life boot-strapped itself by "learning" how to use a larger number of metals for its purposes – first of all for sake of reproduction – parallel to each other and thus caused its onset regardless of the obstacles from the three-functions-role. Concerning the "larger number" just mentioned, whereas most likely few of the metals Mg, Ca, Mn, Fe, Cu, Zn or Mo were used in life's very beginnings for catalytic purposes but the evolving biomass was already capable to select the mentioned metals vs. others like Al, Co or Cd owing to different complex formation constants and changes of the latter due to chemical evolution itself. We restrict our argument to processes **within** some protocell here, yet considering the likelihood that proto- or eobionts dwelled on some mineral interfaces where reductions – possibly in the

[1] Müller-Herold explicitly addresses quantum-mechanical features of emergence in self-ordering of electron spin-controlled (i.e. chemical) systems, using the example of spontaneous ferromagnetic domain formation upon cooling of appropriate materials but the same (topological) argument holds for some sub-volume of the physical space which becomes "suddenly" distinguished by autocatalysis absorbing catalyst(s) from other regions, then using them to reproduce itself, creating and storing (like in ferromagnetic domains) corresponding pieces of information autonomously.

Fe_xS/H_2S system – or photoelectrochemistry (at sulfides like chalcopyrite or at perowskite or molybdates (Bahadur et al. 1958)) took place, providing reactive intermediates for a still highly heterotrophic kind of organism. Then, such minerals going to alter or degrade in this process are to be considered resources for autocatalysis in the protocells additionally, the more so if they contain (thereafter) essential metals like in the examples of photocorroding photoelectrochemical support minerals (Cu, Fe (and S) in chalcopyrite, Ca in perowskite $CaTiO_3$, Mo in molybdates like scheelite). However, metal ion affinities of the ligands thus forming would not suffice to extract Mn, Fe, Zn or even Cu from solutions composed like recent seawater (cp. Fränzle and Markert 2002b). Taking this into account, the emergent step of biogenesis rather occurred in a setting enriched with all the metal salts, like saline springs equilibrating with carbonate minerals or drying pools (but rather not tidal pools, see above) or directly in/at mineral surfaces rather than the open ocean. Although many consider hydrogenases "primordial" for their containing CO and cyano ligands, a palaeoatmosphere with substantial CO would have stopped this sequence of events, in turn suggesting hydrogenases are not as old as often assumed: even allowing for constant replenishment of H_2 in the atmosphere by aqueous Fe(II) photooxidation (Braterman et al. 1983), the steady-state concentrations of dihydrogen would remain very low due to its fast effusion from Earth's rather weak gravitational grip (Lewis and Prinn 1984), that is, levels would also remain too low for becoming a substrate of specialized enzymes. However, if some redoxinert element (which thus would not "see" the difference unless for sulfide precipitation or its end) with hitherto >> 3 functions is partly replaced by another, redox-active one, e.g. Zn by Co in carboanhydrases or nitrile-hydrolases or of Mg by Mn^{2+}, Fe^{2+} in acidic phosphatases, the latter will be established in biomass exactly when changing environmental conditions do separate it from the "alternative" or "ancestor" element biogeochemically (holds for Co as both Mn and Fe are ubiquitous biocatalysts). Among phosphatases, being coupled to redox processes directly via proton pumps at membranes, there is a multitude of both redox-inert (Mg, Zn) and redox-active (Mn, Fe) central ions (Frausto Da Silva and Williams 2001). Anyway, oxidation of the environment necessitated adaptations and modifications of retrieval and transport of all Mn, Fe and Cu.

The Cambrian Revolution not only brought about new animal phyla by the dozens but also saw the advent of (a) hard-bodied animals and (b) the take-over of metazoans hitherto almost hidden among the larger huge-monocell Vendobiota (Seilacher 2008). Thereby, Ca became a prominent bioelement, both in control/organization of cell budding and in formation of hard, calcite-, apatite- or aragonite-based structures like teeth, radulae or outer skeletons. By forming such novel instruments for biting prey or other food large predators like *Anomalocaris* now got into a position of acting as second- or/and higher-level consumers.

Nowadays there are some enzymes which contain Cd or Co instead of Zn (Price and Morel 1990; Frausto Da Silva and Williams 2001). Probably this does not date back to the protocell and its, as shown before, non-random but likewise non-specific uptake of metal ions as there was slight discrimination against both Co^{2+} and Cd^{2+} in the prebiotic salt deposits and oligomers. Moreover there was no role for the corresponding enzymes – carboanhydrases – in prebiotic chemistry: CO_2 most probably was abundant, and uncatalyzed kinetics of CO_2/HCO_3^- interconversion are sufficiently fast to meet the modest demands of any early organism which might have used CO_2 directly. Although Cd (and Ni) are toxic for many quite different organisms now, they are located well inside the "window of essentiality" and Ni is used by all plants also (inter alia in urease); possibly later evolutionary developments caused loss of earlier functions in some cases. These later developments may or may not be linked to formation of metazoans even though then "addition" should prevail, yet might be more than compensated by effects of Gibbs's phase rule in any animal or plant more differentiated than, e.g. a sponge.

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