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Environmental Security

Environmental Heavy Metal Pollution and Effects on Child Mental Development

Risk Assessment and Prevention Strategies

Edited by
Lubomir I. Simeonov
Mihail V. Kochubovski
Biana G. Simeonova

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Environmental Heavy Metal Pollution and Effects on Child Mental Development

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Series C: Environmental Security

Environmental Heavy Metal Pollution and Effects on Child Mental Development

Risk Assessment and Prevention Strategies

edited by

Lubomir I. Simeonov
Solar-Terrestrial Influences Institute
Bulgarian Academy of Sciences
Sofia, Bulgaria

Mihail V. Kochubovski
Department of Water and Communal Hygiene
Republic Institute for Health Protection
Skopje, FY Republic of Macedonia

and

Biana G. Simeonova
Institute of Electronics
Bulgarian Academy of Sciences
Sofia, Bulgaria

Springer

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PREFACE

The book contains the contributions at the NATO Advanced Research Workshop on Environmental Heavy Metal Pollution and Effects on Child Mental Development – Risk Assessment and Prevention Strategies, which took place in Sofia - Katarino, Bulgaria, April 28–May 1, 2010.

A group of specialists of different background participated at the meeting and contributed to the multidisciplinary approach, bringing a wide pallet of opinions and remarks. The spread in specializations of the participants was: air quality, analytical and bioanalytical chemistry, biochemistry, biology and marine ecology, developmental psychology, economics, environmental engineering, environmental medicine, toxicology and risk assessment, and physics.

The goal of the Advanced Research Workshop was to evaluate the existing knowledge on intellectual impairment in children exposed to heavy metals in their living environment. Research needs were identified in order to obtain a clearer picture of the situation in countries and regions in risk, in which the economy is closely related to metallurgy and heavy metals emission and to recommend a strategy for human protection. The importance and need of such evaluation is obvious and timely.

Heavy metals include metals and metalloids with atomic density of 4 g/cm^2 or five times greater than water. They are: arsenic, cadmium, chromium, copper, lead, mercury, silver, zinc, iron, and the platinum group elements, and are of major economic significance. These metals present themselves in various chemical forms and take various routes of exposure; both of these factors can influence the severity of their neurotoxic effects.

Millions of children throughout the world have suffered brain damage as a result of environmental pollution. One of six children is thought to have some kind of developmental disability, but the exact causes are largely unknown. The levels of environmental pollutants, their mixture, their accumulation in humans, and the rising incidence of neurological diseases have been noted recently as a suspicious coincidence. Neurodevelopmental disorders caused by heavy metal exposure are a known contributor to this silent pandemic.

Children exposed to even small amounts of some heavy metals are known to suffer a wide range of neurobehavioral problems, including learning disabilities, memory and attention deficits, impaired problem solving, reduced intelligence test (IQ) performance, increased behavior problems such as aggression, conduct disorder, and criminality, and psychiatric problems such as depression and anxiety. For many pollutants, important questions remain unexplored or have only tentative answers at this time. Of particular importance is the relative importance of prenatal versus postnatal exposure and clarifying the effects of simultaneous or sequential exposure to multiple pollutants. There are also questions about best methods for measuring mental disabilities, such as using IQ tests versus more specific tests of neuropsychological function.

The main objectives of the Advanced Research Workshop were to: to review the principle sources for single and complex mixtures of heavy metal pollutants in the environment; to identify the suitable methodology for chemical analyses in the environment and in humans; to evaluate the existing methods for measuring mental impairment, including their reliability and validity; to recommend a standard testing protocol to be used in future research; to assess the future role of environmental heavy metal pollution in countries and regions in risk and its effects on children's neurological development; to recommend a prevention strategy for protecting children's health and development.

Lubomir I. Simeonov
Solar-Terrestrial Influences Institute
Bulgarian Academy of Sciences
Sofia, Bulgaria

Mihail V. Kochubovski
Republic Institute for
Health Protection
Skopje, FYR of Macedonia

Biana G. Simeonova
Institute of Electronics
Bulgarian Academy of Sciences
Sofia, Bulgaria

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LIST OF CONTRIBUTORS

Alexander Spasov
LABOREX LTD.,
Laboratory of Soil and Waste
15 “Ivan Geshov” Blvd.
1431 Sofia
Bulgaria

Aleksandra Sas-Nowosielska
Institute for Ecology of Industrial Areas,
6 Kossutha Str., 40-832 Katowice
Poland

Amir M.El Shahawy
Occupational and Environmental Medicine Department
National Research Center
El Behoos Street, Dokki, Cairo
Egypt

Anca Diana Oros
Child and Adolescent Psychiatry Department
Obregia Hospital of Psychiatry Bucharest
10 Berceni Str, Sector 4, Bucharest
Romania

Andrzej Kita
Department of Analytical Chemistry
University of Silesia
9 Szkolna Street, 40-006 Katowice
Poland

Anna Dimitrova
Department of Materials Science and Technology
English Language Faculty of Engineering
Technical University of Sofia
8 “St. Kliment Ohridski” Blvd., 1797 Sofia
Bulgaria

António Fiúza
Geo-Environment and Resources Research Center (CIGAR)
Faculty of Engineering, University of Porto
Rua Dr. Roberto Frias, 4200-465 Porto
Portugal

Arman Sargsyan
Faculty of Economics
Yerevan State University of Economics
Nalbandyan St., House 128, Yerevan 0025
Armenia

Biana Simeonova
Institute of Electronics
Bulgarian Academy of Sciences
72 Tzarigradsko Shossee Bul, 1784 Sofia
Bulgaria

Camelia Draghici
Transilvania University of Brasov
29 Eroilor Bldv., 500036 Brasov
Romania

Carmen Dima
Apa Company, Brasov,
13 V. Tepes Str., 500092 Brasov
Romania

Christoph Schmitt
Dr. Schmitt-ISOTOP
Laboratory for Laser Analytical Research
Sauerwiesen 6, 67661 Kaiserslautern-Siegelbach
Germany

Cristina Jelescu
Apa Company, Brasov,
13 V. Tepes Str., 500092 Brasov
Romania

Dimiter L. Tsalev
Analytical Chemistry Department, Faculty of Chemistry
University of Sofia “St. Kliment Ohridski”
1 James Bourchier Blvd., Sofia 1164
Bulgaria

Dragan Gjorgjev
Institute of Public Health of the FYR of Macedonia
50 Divizija No. 6, 1000 Skopje
FYRepublic of Macedonia

Elena Stanescu
National Research and Development Institute
for Industrial Ecology (ECOIND)
Panduri 90-92, Bucharest
Romania

Elisabeta Chirila
Ovidius University of Constanta,
124 Mamaia Bldv. 900527 Constanta
Romania

Eugeniusz Małkowski
Department of Plant Physiology
Faculty of Biology and Environmental Protection
University of Silesia,
28 Jagiellonska Street, 40-032 Katowice
Poland

Fokion Vosniakos
Balkan Environmental Association (B.EN.A.)
Alexander Technological Education Institute of Thessaloniki
P.O. Box 141, 57400-Sindos, Thessaloniki
Greece

Jacek Krzyżak
Institute for Ecology of Industrial Areas,
6 Kossutha Str., 40-832 Katowice
Poland

Jana Petre
National Research and Development Institute
for Industrial Ecology (ECOIND)
Panduri 90-92, Bucharest
Romania

Gabriela Vasile
National Research and Development Institute
for Industrial Ecology (ECOIND)
Panduri 90-92, Bucharest
Romania

George Vasilikiotis
Alexander Technological Education Institute of Thessaloniki
Department of Sciences
P.O. Box 141, 57400-Sindos, Thessaloniki
Greece

Gheorghe Coman
Transilvania University of Brasov
29 Eroilor Bldv., 500036 Brasov
Romania

Gyula Dura
National Institute of Environmental Health
Gyali ut 2-6., 1097 Budapest
Hungary

Hanna Sas-Nowosielska
Department of Plant Anatomy and Cytology
University of Silesia
Jagiellońska 28 Str., 40-032 Katowice
Poland

Konstantinos Nikolaou
OMPEPT - Organisation for the Master Plan and
Environmental Protection of Thessaloniki
Greece

Konstantinos Vosniakos
Alexander Technological Education Institute of Thessaloniki
Department of Sciences
P.O. Box 141, 57400-Sindos, Thessaloniki
Greece

Liliana Cruceru
National Research and Development Institute
for Industrial Ecology (ECOIND)
Panduri 90-92, Bucharest
Romania

Lubomir Simeonov
Solar-Terrestrial Influences Institute
Bulgarian Academy of Sciences
Acad. G. Bonchev Str. Bl.3, 1113 Sofia
Bulgaria

Mahmoud A. Hassanien
Air Pollution Department Medicine Department
National Research Center
El Behoos Street, Dokki, Cairo
Egypt

Margareta Nicolau
National Research and Development Institute for
Industrial Ecology (ECOIND)
Panduri 90-92, Bucharest
Romania

Maria de Lurdes Dinis
Geo-Environment and Resources Research Center (CIGAR)
Faculty of Engineering, University of Porto
Rua Dr. Roberto Frias, 4200-465 Porto
Portugal

Mariana Golumbeanu
International Research Secretariat of B.EN.A.
National Institute of Marine Research and
Development (NIMRD) “Grigore Antipa”
300 Mamaia Bd., 900581 Constanta
Romania

Marta Pogrzeba
Institute for Ecology of Industrial Areas
6 Kossutha Str., 40-832 Katowice
Poland

Mihail Kochubovski
Institute of Public Health of the FYRepublic of Macedonia
50 Divizija No. 6, 1000 Skopje,
FYRepublic of Macedonia

Mirela Coman
Depart. of Mineral Processing, Geology and Environ. Engineering
North University of Baia Mare
62A Dr. Victor Babes Str., Baia Mare
Romania

Momtchil Sidjimov
LABOREX LTD.
Laboratory of Soil and Waste
15 “Ivan Geshov” Blvd., 1431 Sofia
Bulgaria

Péter Rudnai
National Institute of Environmental Health
Gyali ut 2-6., 1097 Budapest
Hungary

Richard Canfield
Division of Nutritional Sciences
College of Human Ecology
Cornell University
302 MVR Hall. Ithaca, NY 14853
USA

Simona Roman
The Health Authorithy of Maramures County
The Public Health Authority of Maramures County
31 G. Cosbuc Str., Baia Mare
Romania

Tamás Pándics
National Institute of Environmental Health
Gyali ut 2-6., 1097 Budapest
Hungary

Thomas A. Lewandowski
Department Health and Nutrition Sciences
Brooklyn College CUNY
4135 Ingersoll Hall
2900 Bedford Avenue, Brooklyn, NY 11210,
USA

Triantaffylos Albanis
Department of Chemistry
University of Ioannina
Panepistimioupoli Ioannina
Greece

Vardan Sargsyan
Faculty of Economics
Yerevan State University of Economics
Nalbandyan St., House 128, Yerevan 0025, Armenia

Yuri Suvaryan
Faculty of Economics, Yerevan State University of Economics
Nalbandyan St., House 128, Yerevan 0025
Armenia

Valery Metodiev
LABOREX LTD.
Laboratory of Soil and Waste
15 “Ivan Geshov” Blvd., 1431 Sofia
Bulgaria

Vasile Oros
Depart. of Mineral Processing, Geology and Environ. Engineering
The North University of Baia Mare
62A Dr. Victor Babes Str., Baia Mare
Romania

Vladimír Bencko
Institute of Hygiene and Epidemiology
First Faculty of Medicine
Charles University in Prague and
General University Hospital
Studnickova 7, CZ 12800 Prague 2
Czech Republic

Włodzimierz Majtkowski
Botanical Garden of National Center for Plant Genetic Resources
Plant Breeding and Acclimatization Institute
National Research Institute
5 Jeździecka Street, 85-687 Bydgoszcz
Poland

ENVIRONMENTAL HEAVY METALS AND MENTAL DISORDERS OF CHILDREN IN DEVELOPING COUNTRIES

MAHMOUD A. HASSANIEN[1]* AND AMIR M. EL SHAHAWY[2]
[1] *Air Pollution Department Medicine Department and*
[2] *Occupational and Environmental Medicine Department,*
National Research Center
El Behoos Street, Dokki, Cairo, Egypt

Abstract. Environmental pollution is a product of urbanization and technology, and other attendant factors of population density, industrialization and mechanization that serve to provide the necessities of the population. For example, in cities of developing countries, the rural-urban migration activated by the search for increased incomes has resulted in the concentration of large populations in relatively small areas under poor conditions of sanitation. Traffic jams and the legendary ‘go-slow’ of automobiles are everyday occurrences in these cities. The impact of pollution in the vicinity of overcrowded cities and from industrial effluents and automobile exhausts has reached a disturbing magnitude and is arousing public awareness. At present, no enough data are available on the extent of environmental pollution because there are no agencies charged with the routine monitoring and protection of the environment. This chapter therefore focuses on the critical issues of heavy metals (HMs) pollution in rapidly developing nations. Once emitted from their sources, they have the property of accumulating in the environment for many years. They enter the human body through inhalation, ingestion, and dermal. They also can accumulate in the bodies of animals and humans before they even cause damage. However; HMs such as Lead (Pb), Cadmium (Cd), Arsenic (As), Manganese (Mn) Nickel (Ni), Chromium (Cr) and Mercury (Hg), are also metabolized in the body in a similar way to nutrient metals. Environmental exposure to HMs can occur through air, soil, drinking water and food stuff. The neurotoxic effects of exposure HMs in the environment, though insufficiently recognized, remains a topic of substantial current concern and interest as it could be considered an early endpoint for health effects induced by exposure to heavy metals. In addition, they can adversely affect the quality of life, and have broad health, social and economic

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* To whom correspondence should be addressed: mhassanien@gmail.com

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implications. Special concern is directed to children as they are the most sensitive population exposed to environmental pollution in general and heavy metals in specific. Finally, the magnitude and potential severity of neurotoxicity problems make it imperative to direct researches towards preventive intervention, and focus on the development of new biomarkers for neurotoxicity at the individual and population levels with emphasis on health education about HMs exposure and their potential for neurotoxicity. The establishment of comprehensive monitoring systems and information gathering should be given priority by governments of the developing countries in the sub-region with support and encouragement from international agencies.

Keywords: Environmental pollution, heavy metals, developing countries, children, mental disorders

1. Main Sources of Heavy Metals

Heavy metals are metallic elements with atomic weights and densities exceeding 5 g/cm^3. Some heavy metals serve as micronutrients at low concentrations as copper (Cu), zinc (Zn) and iron (Fe), but they are only toxic when in excess, while other heavy metals and metalloids as lead (Pb), cadmium (Cd), mercury (Hg), inorganic arsenic (As), Aluminum (Al) and Nickel (Ni) that are toxic even at very low concentrations, hence they are of particular health concern (Schwartz et al., 2010). Those heavy metals have the ability to bioaccumulate in food chain, and children can be chronically exposed to them from different sources as air, water and food leading to their accumulation in body tissues of children and causing various diseases because they act as systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects and they can directly influence behavior and impair mental and neurological functions via influencing neurotransmitter production and utilization (Obiria et al., 2010; Thakur et al., 2010).

LEAD (Pb)

Lead sources can be classified into natural sources and anthropogenic sources; the natural sources result from the natural mobilization of naturally occurring lead from the Earth's crust and mantle due to volcanic activity or weathering of rocks; while, the anthropogenic sources result from mobilization of lead impurities in raw materials such as fossil fuels and other extracted and treated metals; in addition to lead used in products and processes as a result of mining and processing activities, releases from incineration and installations for municipal waste, residues containing lead; and the mobilization of historical lead releases previously deposited in soils, sediments and wastes.

The total global production of lead from mining has downsized from 3.6 million tonnes in 1975 to 3.1 million tonnes in 2004; meanwhile, global refined lead production have increased from 4.7 million tonnes to 7.1 million tones as the recycled lead accounts for an increasingly large part of the supply (UNEP, 2008).

Lead production by developing countries is not much compared to its production by other developed countries as China, Australia, USA and Canada that produce about 76% of the world production. Table 1 shows lead production through different countries including developing ones where, the production via recycling of previously used lead is higher for developed countries (60–70% of their lead production) than developing countries (<30%).

TABLE 1. Lead production from developing countries compared to other developed countries in 2009.

Country	% of global lead production
Peru	9
India	1.5
Mexico	4
Kazakhstan	1
Ireland	1
Poland	1
Morocco	1
Brazil	0.64
China	40
USA	12

Adapted from US Geological Survey- Mineral Commodity Report (2010).

For the past 50 years; the use of organic lead compounds as antiknock motor vehicle fuel additives has been the dominant source of worldwide dispersion of lead into the environment. Although a worldwide phase-out of leaded gasoline is in progress, it is still being used in developing countries all over the world (McCally, 2002). The progressive decrease in lead content in leaded gasoline and providing control measures for lead smelters were the main causes of a decrease in urban lead pollution, for example in Cairo, Egypt, where lead decreased sharply from 1999 to 2002 (EEPP, 2004) and approached nowadays to be less than the standard Egyptian limit values (1 $\mu g/m^3$) (Hassanien, 2009). However, large amounts of fine lead particles still persist in the soil of highly populated cities as city soil, home soil, and as ground contamination near highways and in industrial districts, e.g. Shoubra ElKheima, Egypt (EEPP, 2004). About 60% of worldwide production of lead is used for the manufacturing of batteries (especially automobile batteries), while the remainder is used in the production of pigments, glazes, solder, plastics, cable sheathing, ammunition, weights and gasoline additive. Such industries continue to pose a significant risk to surrounding communities. However, the power storage battery industry may have a relatively low impact on the environment because about 80% of all batteries are recycled (Sarkar, 2002).

In developing countries, there are still remnants of lead paints on walls of old houses, lead pipes or copper pipes welded with lead and surprisingly, this can be seen in areas of old urban housing in developed countries as the USA. Ceramics covered with low-quality leaded paint can also be hazardous as they are still been manufactured in some countries where regulations are not strictly applied. Other more unusual sources of lead exposure also continue to be sporadically found, such as improperly glazed ceramics, lead crystal and certain herbal folk remedies. Children's toys, pencils, furniture and other school tools, also constitute more sources of exposure to lead.

TABLE 2. Heavy metals concentrations in honey from developing countries compared to developed countries (Units in μg/g unless otherwise indicated).

	Pb	Cd	Mn	Zn	Co	Fe	Cu	Reference
Egypt	1.05	0.005	0.63	6.09	0.08	34	1.38	Rashed et al. (2009)
Egypt	4.2	0.01	0.5	9.3	2.5	58	2.3	Rashed and Sultan (2004)
Turkey	–	0.5	0.02	0.06	–	0.27	0.02	Uren et al. (1998)
Poland	0.12	0.016	10.43	19.3	–	9.46	1.37	Bulinski et al. (1995)
Romania	0.2	0	–	6.2	–	10	0.5	Carmen and Cristina (2001)
Italy	141	0.7	19.8	414	–	914	2.7	Caroli et al. (2000)
Spain	–	–	0.133–9.471	1.33–7.82	–	–	0.53	Fernandez-Torres et al. (2005)
Pakistan	0.17	–	0.075	0.3011	0.013	7.433	0.494	Bibi et al. (2008) (Units in ppm)
Saudi Arabia	1.81	–	0.086	0.2110	0.007	5.088	0.023	Bibi et al. (2008) (Units in ppm)
USA	0.24	–	0.064	0.1903	0.012	4.467	0.032	Bibi et al. (2008) (Units in ppm)

Food contamination may be a source of various degrees of exposure to lead. Recently, a study measured lead content in breakfasts and lunches given at a primary school in an area 300 km away from a large city in Santiago, Chile. Each breakfast contained 1.3 μg/g lead and each lunch contained 0.6 μg/g lead, and the sum was 714.1 μg lead/d which exceeds the levels set by the FAO/ WHO experts that mandates 25 μg lead/kg body wt/wk as the maximum level (Bastias et al., 2004). Table 2 shows the concentrations of heavy metals in honey from developing and developed countries.

The mode of food consumption can add more risk as Pb in soil tends to concentrate more in root vegetables (e.g., onion) and leafy green vegetables (e.g., lettuce and spinach); hence, individuals will absorb more lead if their diets contain much of those types of food in addition to enhanced effect if their diets are also deficient in calcium, iron, or zinc. Table 3 demonstrates the levels of cadmium and lead in different foodstuffs of different developing countries compared to the developed ones.

TABLE 3. Levels of Lead and Cadmium in foodstuff of different developing countries compared to developed ones (Heavy metal concentrations are in mg/kg).

	Egypt		Pakistan		Greece	Tanzania		USA		Ethiopia	
Foodstuff	Pb	Cd	Pb	Cd	Cd	Pb	Cd	Pb	Cd	Pb	Cd
Apple	0.19	0.05	0.76	0.14	0.0003	–	–	–	–	–	–
Melon	0.33	ND	0.12	0.26	0.0058	–	–	–	–	–	–
Watermelon	0.30	0.02	–	–	0.0004	–	–	–	–	–	–
Orange	0.15	0.04	–	–	0.0009	–	–	–	–	–	–
Strawberries	0.87	0.02	–	–	0.0019	–	–	–	–	–	–
Banana	0.05	0.02	–	–	0.001			–	–	–	–
Spinach	0.34	0.11	–	–	0.0527	0.37	0.03	–	–	–	–
Lettuce	0.58	0.07	–	–	0.052	0.37	0.03	0.01	0.26	1.02	2.57
Tomatoes	0.26	0.01	1.56	0.33	0.0188	–	–	–	–	–	–
Carrot	0.18	0.01	–	–	0.0141	–	–	–	–	0.08	0.07
Squash	0.42	0.03	1.06	0.33	–	–	–	–	–	–	–
Cucumber	0.19	0.15	1.72	0.36	0.0002	–	–	–	–	–	–
Onion	0.14	0.02	0.06	0.07	0.0032	–	–	–	–	–	–
Garlic	0.12	0.03	0.04	0.09	0.011	–	–	–	–	–	–
Green pepper	0.47	0.05	1.62	0.32	0.0315	–	–	–	–	–	–
Eggplant	0.21	0.02	1.30	0.31	0.0315	–	–	–	–	–	–
Potatoes	0.08	0.02	0.16	0.08	0.0223	–	–	0.01	0.03	–	–

Adapted from Radwan et al. (2006)

ND No Data

Egypt-Radwan et al. (2006); Pakistran-Parveen et al. (2003); Greece-Karavoltsos et al. (2002): Tanzania-Bahemuka and Mubofu (1999); USA-Pennington et al. (1995a, b); Ethiopia-Rahlenbeck et al. (1999)

CADMIUM (Cd)

Natural sources result from natural mobilization of naturally occurring cadmium from the Earth's crust and mantle caused by volcanic activities forest fires and weathering of rocks; while, anthropogenic sources result from the mobilization of cadmium impurities in some materials such as fossil fuels, phosphate fertilizers, and other processed metals – particularly zinc and copper; in addition to releases from cadmium used in products and processes during disposal, recycling, reclamation, incineration or open burning; also it could be released from municipal installations; and the mobilization of historical anthropogenic and natural cadmium releases previously deposited in soils, sediments, landfills and wastes.

In the atmosphere, cadmium occurs attached to particles ranging between 0.5 and 1 µm. The main cadmium species upon emission are oxides, chlorides, sulfides and the elemental form. Oxides (CdO) are emitted by most of the anthropogenic sources. Elemental cadmium is released during high temperature processes such as organic fossil fuel combustion and waste incineration. Sulfides (CdS) are prominent in the emissions from non-ferrous metal production and coal combustion (Kuloglu and Tuncel, 2005; Dillner et al., 2005). WHO has recommended 5 ng/m^3 as an air quality guideline for cadmium in order to prevent any further increases in cadmium levels in agricultural soils (Lazaridis and Colbeck, 2010; Wyrwala et al., 2010).

Foods act as an important source for exposure to cadmium which tends to accumulate in leafy vegetables and kidneys, liver in addition to muscles of animals; hence, people who are eating those foods frequently have a higher risk of cadmium exposure (De Vries et al., 2007). In soil, cadmium mostly remains in the top 15 cm of soil and tends to be retained more in clay soils than sandy soils. There are three main anthropogenic sources of cadmium in the soil: atmospheric deposition, agricultural use of phosphate fertilizers, and use of municipal sewage sludge as a fertilizer. Drinking-water generally contains low cadmium levels, and a value of 1 µg/l or less is often assumed to be a representative content in most situations. Thus, cadmium exposure from drinking-water is relatively unimportant compared with the dietary contribution (WHO/IPCS, 1992); hence, WHO still recommends a guideline value of 0.003 mg/l for cadmium in drinking-water (WHO, 2004).

MERCURY (Hg)

The sources of Mercury can be either natural sources or anthropogenic sources. The natural sources include volcanoes, geothermal activity, wildfires and weathering of rocks; while the anthropogenic releases come from various human activities as burning of fossil fuels, processing ores from mining for gold, zinc, lead and copper, and industrial activities that produce cement and various commercial products, also during incineration of wastes containing such products, then the mercury that has been deposited from the atmosphere can be repeatedly emitted again.

Mercury (Hg) is a metal that is liquid at room temperature and can be found in different forms: (1) elemental mercury (Hg0) which is mostly found in the atmosphere, (2) inorganic mercury in the form of oxidized mercury either in the gas phase or associated with particles and (3) organic mercury which results when mercury combines with carbon, forming simple or large molecules. Organic mercury is most commonly found as methylmercury which can be naturally produced by microorganisms as bacteria and by industrial processing of mercury. The methylmercury is very important as it can bio-accumulate and bio-magnify in the food chain especially fish and shellfish, then it can be a great source of mercury exposure in humans.

Mercury is used in many products, including batteries, paints, switches, electrical and electronic devices, thermometers, thermostat blood-pressure gauges, fluorescent and energy-saving lamps, dental amalgam, pesticides, medications, and cosmetics. Mercury release from dental amalgams (approximately 50% Hg) is a further source of exposure, but in the study of Holmes et al. (2003). Seafood consumption was not the major correlate of baby-hair levels in *control* subjects, with amalgams and medications containing ethylmercury preservative (Rho immunoglobulin, vaccines) playing a more important role. UNEP has estimated a\the stable atmospheric emission of mercury from anthropogenic sources in 2005 by 1930 tonnes as outlined in Table 4 (UNEP, 2008).

TABLE 4. Global anthropogenic air emissions of mercury in 2005.

Region	Emissions (tonnes)
Asia	1281 (66.5%)
North America	153 (7.9%)
Europe	150 (7.8%)
South America	133 (6.9%)
Africa	95 (5%)
Russia	74 (3.9%)
Australia	39 (2%)
Total	1930 (100%)

Adapted from UNEP (2008).

2. Exposure Routes of Environmental Heavy Metals

The routes of exposure to heavy metals for children are namely inhalation, ingestion, skin absorption and trans-placental route. The mode of exposure and the nature of sources usually determines the priority of which route becomes dominant. For example, Lead can be absorbed into the body through inhalation, ingestion, dermal contact or transfer via the placenta. Infants and children are exposed to lead toxicity through different routes depending on local conditions as ingestion of soil and dust contaminated by leaded gasoline and old lead-based paints due to hand-to-mouth activities. Also, when tap-water systems with leaded pipes are used, lead intake via drinking-water can be an important source, while, inhalation exposure may be significant when lead levels in the air are high. Crawling infants are closer to surfaces on which lead dust is likely to be located, a proximity that results in increased exposure both from breathing in the dust and also from ingestion due to hand to mouth activities (Weiss, 2000). It also accumulates in the skeleton of mothers, and its mobilization from bones during pregnancy and lactation causes exposure to fetuses and breastfed infants. Hence, the lifetime exposure of women before pregnancy is important.

House dust is potentially an important and persistent source of cadmium exposure in areas with contaminated soils, especially where driveways have been covered with residues from non-ferrous metal production (e.g. zinc ashes or sintels as oven sludge), a condition that can affect children more than adults especially during the early years of life where they stay for longer durations in their homes. Passive smoking represents an additional source of cadmium where fathers and mothers who smoke cigarettes are exposing their children to a great risk of cadmium exposure which may exceed that from food (Erzen and Kragelj, 2006).

Recent data about cadmium exposure indicate that food is the main source of cadmium exposure in the general population especially children, being responsible for 94–99% of the total intake in non-smokers (Wennberg et al., 2006). The provisional tolerable weekly intake (PTWI) for cadmium was set since 1989 by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) as weekly intake of 7 μg/kg body weight (kgbw), while the USEPA Reference Dose amounts to 1 μg/kgbw/day in food and 0.5 μg/kgbw/day in drinking-water (ATSDR, 1999; Horiguchia et al., 2004).

3. Children's Vulnerability to Environmental Heavy Metals

Children and infants undergo continuous rapid growth and development; hence, they have vulnerability windows in many periods which make them exceptionally susceptible to harmful effects of various chemical and physical agents. During fetal period, rapid cell growth and primary differentiation occurs, giving more chances for toxicants to cause congenital anomalies or mutations (Selevan et al., 2000). Also, during the first years of life, structures are still being developed as the nervous system which has a limited capacity to repair structural damages. If any destruction of brain cells occurred by chemicals such as lead and mercury, or failure to establish vital connections between nerve cells, it may result in permanent neuro-developmental damage (Rice and Barone, 2000). The whole sequence of metabolism and detoxifiaction of toxic compounds in children is immature and less efficient than that of adults, particularly during infancy, so, they are more susceptible to hazardous effects than adults and drugs prescribed for them have lower doses per kg of weight (Faustman et al., 2000; Scheuplein et al., 2002).

Surprisingly, children are more heavily exposed, per unit of body weight or body surface, to environmental risk factors than are adults. As infants and young children breathe more air than adults, drink more water and fluids and eat more food and in relation to their body weight as shown in Table 5.

TABLE 5. Child and adult intakes from air, water and food.

Medium (unit)	Child (<1 year)	Adult	Ratio (child/adult)
Air (m^3/kg-day)	0.44	0.19	2.3
Water/Fluids (g/kg-day)	161.0	33.5	4.8
Food (g/kg-day)	140.0	23.0	6.1

Adapted from Licari et al (2007)

The absorption of chemicals in the intestines is greater in children than adults. For example, infants absorb about 50% of the lead content in food, while adults absorb only 10% (Valverde et al., 2002). Moreover, children have different dietary choices and food preferences than adults. For instance, children usually consume much more milk and dairy products. The infants and toddlers show exploratory behavior and hand-to-mouth activity, exposing them to much higher quantities of toxicants that typically concentrate in dust and soil, such as lead and other chemical compounds (WHO, 2007). Children have more years of life ahead of them than do adults, so they have more time to develop chronic diseases that take several decades to appear.

Air pollutants provide an important example of children's exposure. Young children often spend most of their time at home and usually crawling on the floor. The surface of floors and the layer of air near the floor are major sources of chemical agents, including compounds in environmental tobacco smoke (ETS), radon, and combustion products from heating and cooking devices, as well as outdoor air pollutants, such as particulate matter which contains many hazardous pollutants, heavy metals one of them. Children also have increased air intake per kg of body weight, added to the higher susceptibility of their developing lungs to air pollutants which finally lead to higher risk of health hazards (Bruce et al., 2000; Ezzati and Kammen, 2001).

4. Biochemical Mechanisms of Heavy Metals Toxicity

The toxicity of heavy metals from the biochemical mechanisms point of view show different ways according to the nature of metal exposure, physiological status, and biochemical reactions in the human body. For example, Lead (Pb) affects different enzymatic and metabolic pathways in a unique way; it primarily interferes with the pathway of heme biosynthesis; it inhibits the δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase enzymes via binding strongly to their sulfhydryl groups; hence, the ALA and protoporphyrin IX will accumulate in blood and their concentrations will increase in urine, blood and other tissues. The ALA also competes with a neurotransmitter named γ-aminobutyric acid (GABA) causing the production of reactive oxygen species (ROS) that destroys GABA receptors and finally ends by triggering behavioral disorders in exposed population especially children.

Sometimes, neurological manifestations may resemble those of acute intermittent porphyria (AIP) if high levels of lead (>60 µg/dL) and ALA (>1 µM) in the blood of exposed children were detected (Adhikari et al., 2006). However, it was found that Pb depresses coproporphyrinogen oxidase, resulting in increased coproporphyrin in urine (CP). In addition, Pb also interferes with the normal functioning of the intramitochondrial enzyme ferrochelatase, which chelates iron via protoporphyrin. Failure to insert Fe into the protoporphyrin ring results in depressed heme formation and an accumulation of protoporphyrin; this in turn chelates zinc in place of Fe, to form zinc protoporphyrin (Figure 1). These effects also result in modifications of some other metabolite concentrations in urine (ALA-U), blood (ALA-B) and plasma (ALA-P). Moreover, the activities of pyrimidine nucleotidase (P5´N) and nicotinamide adenine dinucleotide synthase (NADS) are also modified in blood after Pb exposure.

Lead interferes with endogenous opioid system and efficiently breaks the ribosyl phosphate group of tRNA. It can also mimic the activity of calcium, thus competing with calcium and zinc ions in finger proteins dependent on these metals (Olympio et al., 2009). In the same vein, cadmium interferes with various important mechanisms as differentiation, proliferation of cells and gene expression. Cadmium causes oxidative damage that affects the DNA, proteins and lipids of cell membrane. The induction of oxidative damage is associated with mitochondrial dysfunction, deregulation of intracellular antioxidants and apoptosis. Oxidative damage to DNA can finally lead to mutations which cause cancer.

Exposure to Pb, Cd or both of them together was suggested to disrupt the cholinergic functions in CNS which appears as decreased activity of acetylcholinesterase (AchE) and decreased acetylcholine turnover rates. This process seems to occur via altering the intracellular calcium metabolism and impairing calcium function as a 2nd messenger in the CNS (Hassanien, 1991; De Castro et al., 1996; Antonio et al., 2003; Khan et al., 2009).

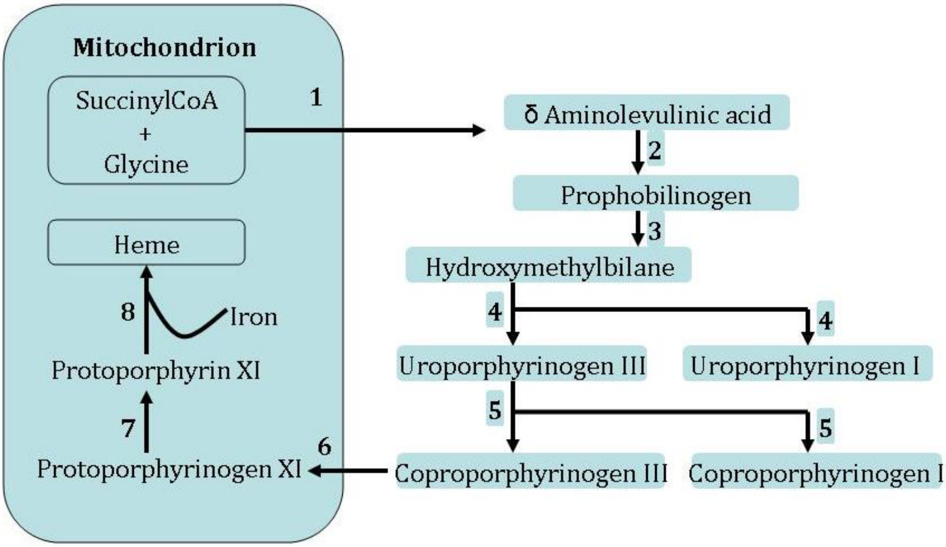

Figure 1. Simplified pathway of heme synthesis in humans (adapted from Daniell et al. (1997)).

5. Mental Health Hazards of Heavy Metals Toxicity

The general body of literature on lead toxicity indicates that, depending on the dose, lead exposure in children can cause a wide spectrum of health problems. Children (and developing fetuses) appear to be particularly vulnerable to the neurotoxic effects of lead. Well-designed prospective epidemiologic studies has frequently demonstrated that even low-level lead exposure in preschool children (with blood lead levels in the 5–25 µg/dL range) can result in deficits in intellectual development as manifested by lost IQ points; hence, the Centers for Disease Control (CDC) in US has lowered the allowable blood lead level in children’s blood from 25 to 10 µg/dL and recommended universal blood lead screening of all children between the ages of 6 months and 5 years. Figure 2 summarizes the main health effects at different blood lead levels in children.

In children, chronic exposure to lead causes a slowing of growth, hearing impairment, neurobehavioral alterations, decrease in the intelligence coefficient (IQ), anemia, decreased 1,25-dihydroxy vitamin D3 plasma levels and action, slowing of myelin fiber nerve impulse, intestinal colic and associated gastro-intestinal symptoms, and encephalopathy. In contrast to adults, the effects of chronic exposure to lead in children are irreversible, even after a decrease in blood levels (Wang et al., 2009).

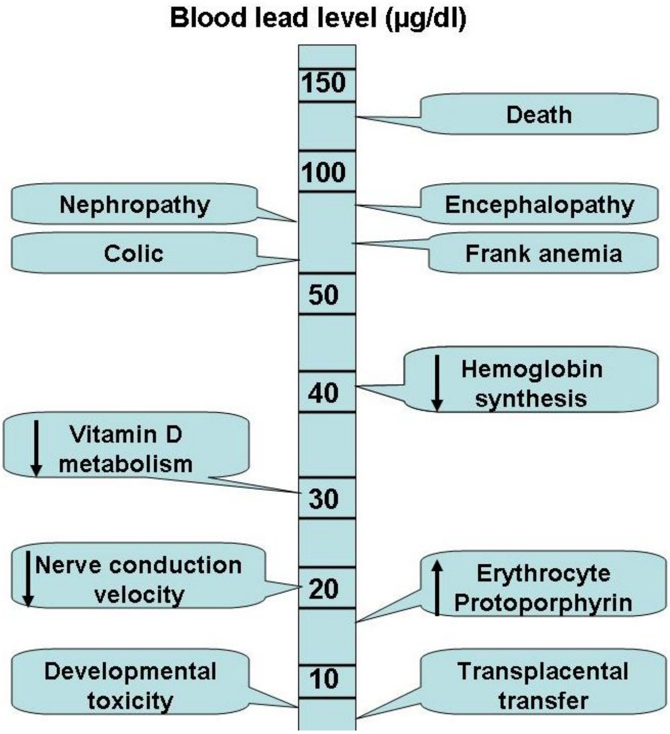

Figure 2. Health effects with different blood lead levels in children and adults.

Lead exposure during pregnancy may lead to congenital anomalies as hemangiomas and lymphangiomas, hydrocele, minor skin anomalies, and undescended testicles. It is also associated with low infant body weight at birth. Pregnant mothers that were previously exposed to lead, usually store increased concentrations of lead in their bones. Such mothers, during pregnancy or breastfeeding, require additional calcium that is released from bone reserves together with lead leading to increased lead levels in mother's blood then lead is transferred through placenta or breast milk to the fetus or child during the period of life when it is most sensitive to lead. Among the delayed effects induced by this mechanism and evident during childhood, adolescence, or adult life, are neurobehavioral alterations such as decrease in the intelligence coefficient (IQ), aggressive behavior, and a tendency to drug abuse and to delinquent behavior (Tchernitchin et al., 1999).

However, prolonged exposure to low levels of cadmium affects mainly the kidneys as cadmium irreversibly damages the proximal tubules of each nephron which is the functional unit of the kidney. This is first manifested by leakage of low molecular weight proteins (LMWP) and essential ions, such as calcium, into urine, with progression over time into renal failure. There are also many studies related to Cd and cholinergic enzymes which are responsible for signal transmission from brain to other parts of the body (Hassanien, 1991).

6. Neurotoxicity of Children due to Heavy Metals

The nervous system is one of the most sensitive and comprehensive systems of the body. Its damage is usually irreversible or controllable, so, it deserves more attention than many other chronic diseases. Unfortunately, as a result of industrialization, there has been increasing exposure of children to neurotoxic chemicals especially heavy metals as lead, mercury, cadmium and manganese which are considered the most prominent neurodevelopmental threat. It is worthy to note that neurodevelopmental damage doesn't necessarily show up as a clinically obvious condition, but, it includes the whole spectrum of decreased function which is termed *sub-clinical toxicity.* Widespread sub-clinical neuro-toxicity can affect the health, well-being, intelligence and even the security of an entire nation. Recently, it was confirmed that early exposure to neurotoxins could increase risk of degenerative diseases, as Alzheimer's and Parkinson's disease, in Later Life, and it was estimated that the total costs of neurobehavioral diseases of environmental origin in children were $18.4 billion in 2006 in USA (Nambudripad, 2007).

Lead is a well-known neurotoxin and over the past 60 years, there has been increasing research on the neuro-developmental effects of lead and growing public awareness of the dangers of lead to children; however, governmental regulations are still lagging behind scientific knowledge as there are many new cases of toxicities that occur every year. Children are more susceptible to the neurotoxic effects of lead for many reasons; children, in comparison to adults exposed to similar dose, (a) absorb more lead, (b) excrete proportionally less of the absorbed lead and retain more lead in their bodies, (c) deposit relatively more of the retained lead in the brain, (d) their developing brains are more damaged by lead than mature brains as human brain development continues well after birth into the teenage years and, for certain systems, into the 20s of life (Giedd, 2004).

The threshold for blood lead level to produce lead toxicity in children is lower than that of adults, it was 60 µg/dl in 1960s, lowered to 40 µg/dl in 1970s, 30 µg/dl in 1975, 25 µg/dl in 1985, 10 µg/dl in 1991, then Centers for Disease Control and Prevention (CDC) reported that they no longer consider any blood lead level to be safe for children. Precise follow up of the threshold for lead toxicity in children and its changes over time will direct our thinking into this fact "there is no threshold for neurobehavioral effects in children exposed to lead" (Chiodo et al., 2004). In many areas there have been major decreases in

B-Pb levels in recent decades, mainly because of the phasing out of leaded petrol but also because of reductions in other sources of exposure. At present, the lowest average B-Pb level in several European countries is about 20 μg/dl, but reliable B-Pb information from many parts of the world are still lacking.

Mercury (Hg) is a potent neurotoxic chemical. Unborn children (i.e. fetuses) are the most susceptible population group, the exposure being mainly from fish in the diet of the mother. Hg is also excreted in mothers' milk. Human biomonitoring and diet-modeling data indicate that tolerable dietary intakes of Hg in the form of methyl mercury are exceeded among subpopulations that consume large amounts of fish, e.g. in Scandinavia, North America and France. For several species of (mainly large predatory) freshwater and marine fish and mammals, a mercury level of 0.5 mg/kg, the value used as a guideline in many countries, is often exceeded.

7. Autism and Environmental Heavy Metals

Mental and neurobehavioral disorders in children especially autism and autistic spectrum disorders (ASDs) are becoming more and more common, which draws attention that something in the environment is at least partly responsible. Many studies have reported elevated levels of heavy metals especially lead, mercury, cadmium and arsenic in urinary samples, hair samples and teeth of ASDs children. The relation between heavy metals toxicity and neurobehavioral disorders was also confirmed by improvement of children suffering from those disorders after being treated with chelation therapy which mobilizes the heavy metals out of the body, thus, decreases the body burden of heavy metals, and many researchers have confirmed that heavy metal removal by chelation is associated with partial remission of ASD behaviors (Lonsdale et al., 2002; Holmes, 2003).

Many studies investigated a possible link between lead exposure, ASD, and childhood neurodevelopmental disorders with reported elevated blood lead levels in ASD children beyond the normal range (Kumar et al., 1998). A study on a group of Canadian children with an unspecified ASD-related disorder revealed elevated urinary levels of lead and other heavy metals, those children were treated with cuprimine (a metal-mobilizing treatment) and metal removal had significantly improved the children's behavior, which confirms the role of heavy metals. Other heavy metals could contribute. As evidence of excessive exposure to lead, mercury, cadmium and arsenic has been reported (Audhya, 2004). Superficial resemblances between mercury poisoning and ASD prompted the suggestion that mercury might also be causally involved (Bernard et al., 2001). Another study, in Kuwaiti children, reported significant elevations of metals in the hair of ASD children versus controls – lead (Pb) was two-fold elevated, while mercury (Hg) levels were 15 times higher than in controls (Fido, and Al Saad, 2005).

Due to the noted shortage of these studies in developing countries, these countries should learn from the experience of developed ones to avoid the problems of HMs in the environmental media and their effects. Mercury was linked to the occurrence of autism; analysis of hairs and teeth of ASD children showed total mercury levels were over three-fold its levels in control children. Another recent study examined the association between autism prevalence in 1184 school districts in Texas and local environmental release of mercury. The study concluded that for each 0.5 t of environmental mercury release (determined by the US Toxic Release Inventory) there was about 61% increase in the rate of autism (Palmer et al., 2006).

Researches on arsenic have also found a link when significant quantities of arsenic were released in a clinical trial of heavy metal removal in ASD with the chelation agent thiamine tetrahydrofurfuryl disulfide (TTFD); increases in urinary cadmium, nickel, and lead were observed (Lonsdale et al., 2002).

8. Genetics as a Major Determinant of Neurotoxicity by Environmental Heavy Metal

Genetics play an important role in every health aspect of our lives, however; the link between genetics and environmental exposures especially to heavy metals has not been fully revealed yet. There are evidences that genetic mechanisms can determine the outcome of exposure according to two levels of interference, first is the genetic predisposition of exposed child, and second is the genetic mechanism by which the heavy metal can cause toxicity and in particular the neurotoxicity.

Heavy metals have different genetic mechanisms by which it can cause neurotoxicity. For example; lead can displace zinc from its binding sites in DNA proteins and receptor channels thus altering the regulation of genetic transcription. Lead can also accumulate in cell nuclei and combine with nuclear proteins and chromatin. It can also cause deleterious effects on gene regulatory proteins which regulate gene expression, signal transduction, cell growth and differentiation, and chromosome structure (Zawia et al., 2000; Crumpton et al., 2001).

Environmental heavy metals, including Pb, Mg, Cd and As were also associated to another genetic mechanism for toxicity which is the DNA methylation that is important for the processes of neurodevelopment (McVeigh et al., 2001; Bleich et al., 2006; Dolinoy et al., 2007). Metals are known to increase the production of reactive oxygen species (ROS) (Fowler et al., 2004) and oxidative DNA damage can cause alterations in methylation metabolism which in turn could initiate a vicious circle of epigenetic phenomena including gene-specific DNA hypo- or hypermethylation, resulting in aberrant gene expression and also in diminished glutathione activity causing the cells to be more vulnerable to oxidative stress (Chen et al., 2001; Takiguchi et al., 2003; Chen et al., 2004; Valinluck et al., 2004; Chanda et al., 2006; Reichard et al., 2007). Finally, there is conflicting evidence that epigenetic phenomena may relate between early life exposure and late onset of disease, hence; further research in heavy metals should study the role of epigenetics in determining late-onset health effects from heavy metal exposure (Wright and Baccarelli, 2007).

9. Health Risk Assessment (HRA) of Neurotoxicity due to Heavy Metals in Children

Nervous system is a very sensitive body system, and this sensitivity is increased in children due to immaturity of their nervous mechanisms, hence; the manifestations of neurotoxicity in children are exceptionally important as it can be used for early detection of environmental exposures especially the long term exposures to low levels of heavy metals. This can be done using different well established techniques that can be collectively used for health risk assessment of exposed children. Starting from the source of pollution there should be biomonitoring of food sources for elevated levels of heavy metals that will be consumed by people and especially children.

Lead exposure in children can be monitored via different well established methods that vary in their efficiency and credibility and the most reliable marker used for biomonitoring is lead concentration in whole blood (BPb); other biomarkers for Pb exposure exist but they have some limitations and their accuracy needs to be improved. There is plenty of scientific data that confirm the continuous mobilization of Pb from bone back into blood that could takes years; a study conducted on children by Roberts et al. (2001) reported that the children had BPb levels 25–29 μg/dL and were left without treatment by chelation, their BPb required about 2 years to decline to <10 μg/dL. So, single BPb will not be sufficiently sensitive and it is highly recommended to do serial BPb measurements for children to give more accurate index of long-term Pb levels and possible health outcomes, Table 6 outlines blood lead levels from different developing countries.

The toxic effects of Pb are primarily associated with fraction of lead that exists in plasma (plasma-Pb) as this fraction is the most rapidly exchangeable

TABLE 6. Blood lead levels in children from different developing countries.

Country	Blood Lead Level (BLL)	Children from	Reference
China	63.88 μg/L (Mean BLL)	Children under 7 years old in Chengdu-China	Jiang et al. (2010)
Egypt	3–28 μg/dL	Children living in high-risk areas for lead pollution	Mostafa et al. (2009)
Egypt	4.817 ± 2.97 μg/dL	Children from greater Cairo	Sharaf et al. (2008)
India	15.11 ± 5.62 μg/dL	Children residing in a village 0.5 km from a zinc–lead smelter	Hedge et al. (2010)
Uruguay	9.0 ± 6.0 μg/dL	Children in neighborhood of Montevideo city	Queirolo et al. (2010)
Kenya	3.3–24.7 μg/dL	Children under five years in Kibera slums in Nairobi	Olewe et al. (2009)

element in the blood compartment; hence, monitoring the concentration of Pb in plasma (or serum) has recently gained increased attention (Fernando et al., 2005). Moreover, Pb level in saliva was also proposed as an easy method of Pb detection and monitoring; but, it has shows variations from BPb levels and Plasma Pb and it needs more complex equipment for analysis as it shows very low levels of Pb; hence, it is not considered a reliable biomarker.

Hair samples are another easy technique to take samples from children; about 30–40 strands of clean non stained hairs are taken from the scalp of the child then prepared and analyzed for heavy metals. This technique can provide a detailed overview of heavy metals to which the child was exposed (Wright et al., 2006). Hair is an ideal target for biomonitoring as it is easily collected from children without any invasive techniques and with no cost in addition to easy storage and transportation of collected samples to the laboratory to be analyzed. So, it was firstly proposed to be the most suitable method especially for developing countries; but, unfortunately, it has many limitations. It can't differentiate between endogenous Pb and exogenous Pb even after taking care during sample preparation by washing the hair in water and acetone. Moreover, there are many confounding factors that affect its level as age, sex and hair color, in addition, there no established reliable data on baseline hair contamination levels in the children which makes it very difficult to decide if the detected levels are high or low than normal levels.

Nails have recently been recognized as an invaluable tissue for monitoring children environmental exposure; it can provide a good indication of exposure to many heavy metals over a period of time. The nails are considered superior to hairs as they are easily collected from children without invasive techniques and with no cost in addition to easy storage and transportation of collected samples to the laboratory to be analyzed and moreover, they have slower growth rate than hairs which helps to provide more integration of Pb exposure (Nowak and Chmielnicka, 2002; Samatha et al., 2004). Toenails are usually preferred as they are not largely affected with exogenous Pb and they are 50% slower to grow than fingernails. So they are usually high variations between Pb levels in the toenails and fingernails of the same subjects; hence, it isn't considered a reliable method for assessing Pb exposure.

Bones are very important as they account for 70% of body burden of Pb in children; hence, bone-Pb is used for assessment of cumulative or long term exposure. It is done via non-invasive X-ray fluorescence (XRF). But, its levels vary greatly between different bones; and in population exposed to low-level Pb as young children, the calculated bone-Pb level shows very high variability which makes it almost a non sensitive biomarker (Ambrose et al., 2000; Todd et al., 2001). Tooth lead (TPb) in children is considered a valuable biomarker over bone-Pb as teeth accumulate Pb for long durations as bone, but they are more accurate than bone as the Pb losses from teeth are much slower; moreover, exfoliated primary teeth from children after the age of 6 years are easy to collect and analyze. Table 7 shows the levels of tooth-Pb that differs according to the layer of tooth, where Pb level in the whole tooth differs from dentine and enamel levels of the same tooth, with the most accurate of them in children is the dentine level as it provides evidence of exposure during the early childhood years (Gomes et al., 2004).

Neuropsychological evaluation of children especially school children in polluted areas could provide important tools for assessment of their neurotoxicity status at the earliest stages, it should include tests for Intelligence Scale, Visual Motor Ability, Language, Memory, Learning and psychological status (Gioia et al., 2000; Wright et al., 2006).

TABLE 7. Lead and cadmium levels in children from developing countries.

Country	Levels	Children from	Reference
Turkey	2.41 ± 2.22 µg/g (Hair lead level)	School children exposed to smoking and highways near schools	Tulin et al. (2007)
Turkey	53.38 ± 44.13 µg/g (Hair Cadmium level)	School children exposed to smoking and in highways near schools	Tulin et al. (2007)
Turkey	1.37 ± 0.66 µg/g Tooth lead levels (TPb)	Children from Ankara (large city with heavy traffic)	Karahalil et al. (2007)
India	33.68 ± 22.59 µg/g Tooth lead levels (TPb)	Children residing in a village 0.5 km from a zinc–lead smelter	Hedge et al. (2010)

10. Conclusions

Children are our future; their health and wellbeing should take the highest consideration in any long term plan on the national or international scale. It is highly recommended that long term actions should be implemented to decrease the burden of environmental heavy metals to which children are continuously exposed.

The recommended actions for mitigation and future prevention of this problem could be concentrated into 6 main themes: (1) more strict regulations on heavy metals, (2) Enhanced environmental monitoring of levels of heavy metals worldwide, (3) wide scale biomonitoring and screening for the levels of heavy metals in children and mothers, (4) health education programs at all scales (schools, mass media and political sector), (5) acceleration of decontamination efforts in polluted areas, (6) current research direction towards prevention of further exposure of children to the effects of heavy metals pollution.

The monitoring of levels and trends in metals pollution world-wide and in developing countries in particular should be a priority; because, there no agency or database exists to give us exact levels and trends in pollution by heavy metals. However, there are some monitoring of the use and distribution of worldwide exposure trends (e.g., leaded gasoline), but the sources of exposure to heavy metals are various, making it difficult to infer the impact of such exposure trends on exposure at the individual scale. A more precise picture would be available if regular sampling and analysis of heavy metal levels in air, soil, drinking water, and food were done using standardized methods.

Wide scale biomonitoring of exposure to heavy metals in the form of population based studies should be implemented in coordination with international organizations. Such an effort should be well organized and funded to standardize and unify protocols for defining test populations that are fairly representative of national/regional trends in addition to sampling techniques and analysis of samples. Although similar surveys appear regularly in the literature, but they lack the global view and usually have different methodologies which make it very difficult to arrange them into a unified framework that could be used to direct future plans to mitigate the effects of heavy metal pollution all over the world.

Health education about heavy metals, their health hazards and how to protect new generations from its impacts; governments, scientists, and the general public should be aware of this knowledge. As such information is not easily accessed or grasped by average citizens and policy-makers, also, it is usually taught on a very small scale in schools of medicine, nursing, or other allied health professions. Formal education programs should contain information about health and environmental effects of pollutants especially lead and other heavy metals, to change human behavior for better prevention and mitigation.

There should be long term international agreements that regulate the production and commerce of products containing hazardous components as heavy metals. Products containing lead for instance, should be prohibited or regulated in the countries of their origin. Current scientific and medical researches (and funding) are giving the priority for studying chronic diseases as heart diseases, neurologic diseases, and cancer. So, it is more practical to increase attention towards research on prevention, and to investigate the various risk factors for the diseases of interest as research on the impacts of heavy metals on human health especially when we take into consideration the amount of unresolved issues in this field of knowledge.

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EXPOSURE ASSESSMENT TO HEAVY METALS IN THE ENVIRONMENT: MEASURES TO ELIMINATE OR REDUCE THE EXPOSURE TO CRITICAL RECEPTORS

MARIA DE LURDES DINIS* AND ANTÓNIO FIÚZA
Geo-Environment and Resources Research Center (CIGAR), Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal

Abstract. The anthropogenic sources for heavy metals include mining, industrial productions, untreated sewage sludge and combustion by-products produced by coal burning power plants. Atmospheric emissions are probably the most preoccupant to human health and to the environment due to either the great quantity involved, or their widespread dispersion. Mercury, lead and cadmium represent a great issue, mostly due to their ability to travel long distances in the atmosphere before deposition. Exposure assessment to emissions of heavy metals implies to identify and quantify the sources, how heavy metals may be dispersed in the environment and which adverse effects they might cause on human health and on the ecosystem. Heavy metals emissions and releases into the environment are available at the European Pollutant Release and Transfer Register (E-PRTR, 2010). In this data base main sources from 27 European countries are identified. Data concerning Cd, Hg and Pb were extracted for the year of 2007. This work focuses on the review and analysis of the principal sources of heavy metals emissions into the environment and their role in critical receptors' environmental exposure. The review of the principal sources may give a clearer picture of the situation: the potential regions at risk and the extension of the contamination, essential to the exposure assessment by critical receptors. On the other hand, exposure assessments studies and consequent research allow a better knowledge of heavy metals sources, emissions, pathways and fate in the environment. Therefore both a complete exposure assessment and a consequent risk assessment are needed to establish the identification and implementation of measures to eliminate or to reduce the exposure.

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*To whom correspondence should be addressed. e-mail: mldinis@fe.up.pt

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1. Introduction

By definition, heavy metals are any metallic chemical element that has a relatively high density (superior to 5 g/cm^3); most of them are toxic or carcinogenic even at low concentrations, such as mercury (Hg), cadmium (Cd), arsenic (As) and chromium (Cr).

Poisoning by exposure to heavy metal is well known to affect central nervous function, damage blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure can cause slower progressing physical, muscular, and neurological degenerative processes. Allergies may also occur and repeated long-term contact with some metals, or their compounds may become carcinogenic.

Heavy metals in the environment may result from many different activities and sources and may enter into the environment by a wide range of processes and pathways; heavy metals are naturally found on earth's crust and have been used in many different applications over decades.

Generic sources of heavy metals include mining and industrial production such as foundries, smelters, oil refineries, petrochemical plants and chemical industry, untreated sewage sludge, disperse sources such as metal piping, traffic and combustion by-production from coal burning power plants. Atmospheric emissions are probably the most harmful to the environment, and consequently, to human health due to either the great quantity involved, or their widespread dispersion which may originate many different exposure pathways. In particular, these three heavy metals (Hg, Pb and Cd) are of great concern to human health and to the environment, mostly due to their ability to travel long distances in the atmosphere before deposition.

Lead is a very soft metal and over decades has been used in many different applications: building materials, pigments for glazing ceramics and pipes for transporting water. It is classified as number 2 in ATSDR's (Agency for Toxic Substances and Disease Registry) top 20 list (ATSDR, 2007). During the last century, the lead content in fuels was a main source of pollution to the environment and, particularly, the lead released to the atmosphere was especially hazardous to children. Today most of the 25 millions of tons of lead produced every year is used in batteries.

Cadmium is number 7 in ATSDR's top 20 list (ATSDR, 2008). It is widely used in batteries and in paint pigments. Cadmium is also a by-product of the mining and smelting of lead and zinc. It is a common component of commercial fertilizers used in agriculture (insecticides and fungicides) and is frequently found in agricultural soils. Cigarettes also contain cadmium.

Mercury is naturally generated from volcanoes. It is also a naturally occurring metal used in many man-made products and processes. It may appear in three different forms: (i) organic mercury (predominantly methylmercury, ethylmercury and phenylmercuric acetate); (ii) non-elemental forms of inorganic mercury (salts such as HgS, HgO, $HgCl_2$); (iii) elemental mercury, in the form of a thick liquid at room temperature, mostly familiar by the silver liquid inside mercury thermometers (EPA, 2006). Mining and paper industry are significant producers of mercury. Mercury is number 3 in ATSDR's top 20 list (ATSDR, 1999). Until 1990 mercury was commonly added to paint as a fungicide; today it can be found in thermostats and in some medicines (mercurochrome and merthiolate) and also in vaccines. Mercury is still being used in gold mining in some parts of Latin America and Africa.

Heavy metals emissions, sources and quantities from 27 European Member states as well as Iceland, Liechtenstein and Norway, are registered at the European Pollutant Release and Transfer Register website (http://prtr.ec.europa.eu/Home.aspx). In these inventory heavy metals sources or sectors are identified and detailed air emissions are available. Data regarding the heavy metals of concern in this study were extracted from the E-PRTR inventory for the year of 2007. This work focuses on the review and analysis of the principal sources of heavy metals emissions into the environment and their role in critical receptors' environmental exposure. The review of the principal sources may give a clearer picture of the situation, in particular, the potential regions at risk, the extension of the contamination (in particular for Hg, Cd and lead emissions) and the exposure assessment by the critical receptors. Consequently, measures to eliminate or to reduce the exposure may be identified and implemented.

2. Heavy Metals Occurrence and Exposure

2.1. CADMIUM NATURAL AND ANTHROPOGENIC SOURCES

Cadmium is classified as human carcinogenic group I. It occurs naturally in the environment together with Zn, Pb and Cu and is present on the earth's crust between 0.1 and 0.5 ppm, although higher levels may accumulate in sedimentary rocks. Marine phosphates have also been referenced as containing levels up to 500 ppm (Cook and Morrow, 1995; WHO, 1992). Cadmium is also present in volcanic activities and forest fires which have been reported as natural sources of cadmium air emissions (Jarup, 2003). Cadmium releases to the environment may result either from these natural sources, or from anthropogenic sources. Anthropogenic sources of cadmium and their compounds may be grouped in the following sectors (E-PRTR, 2010): (i) Energy sector (mineral oil and gas refineries, thermal power stations and other combustions installations); (ii) production and processing of metals; (iii) Mineral industry (production of cement clinker or lime rotary kilns or other furnaces, manufacture of glass fibre, manufacture of ceramic products including products tiles, bricks, stoneware or porcelain); (iv) Chemical industry (industrial scale production of basic organic and inorganic chemicals, production of phosphorus nitrogen or potassium based fertilizers); (v) Waste and waste water treatment (disposal or recovery of hazardous waste, incineration of non-hazardous waste included in Directive 2000/76/EC-waste incineration); (vi) Paper and wood production processing; (vii) Animal and vegetable products from the food and beverage sector.

Cadmium is not mined but it is a by-product of the smelting of other metals such as zinc, lead, and copper. People living near to any of these types of industry may be exposed to cadmium.

Cadmium is used in fertilizer applications and sewage sludge to farm land. More or less intensively these sources will lead to the contamination of soils, increasing Cd intake by crops and vegetables for human consumption. Food stuffs are the major source of Cd in non-smoking population (it represents more than 90% of human exposure to Cd), its concentration by these pathways is highly variable and the intensity of the exposure depends on dietary habitat (ENHIS, 2007). Cadmium compounds are used in PVC stabilizers (essential additives in processing PVC resins), colour pigments, several alloys and more commonly in Ni-Cd batteries (Jarup, 2003).

Products containing cadmium are rarely recycled, being usually dumped together with household waste, thereby being dispersed in the environment, especially if the waste is incinerated (Jarup, 2003). As seen before, the incineration of cadmium products will lead to higher cadmium emissions to the environment, in particular to air. Near industrial areas or hazardous waste sites this will occur more intensively and consequently higher levels of Cd may be present in soil. In addition, car exhaust may contribute to cadmium concentration in soils near roads. Low levels of dissolved cadmium may also be present in surface waters.

Exposure to cadmium may occur through ingestion of terrestrial food, cultivated in contaminated soils or contaminated from atmospheric deposition, contaminated water and from inhalation. Nevertheless, most of the terrestrial food contamination comes from cadmium uptake by plants from fertilizers, sewage sludge and atmospheric deposition.

Exposure at low levels usually does not produce immediate health effects, but may cause severe health problems over long periods. Cadmium is toxic to humans, animals, micro-organisms and plants, however only a small amount of cadmium intake is absorbed by the body and will be stored mainly in bones, liver and, in case of chronic exposure, in kidneys. In the last few years there have been some evidences that relatively low cadmium exposure may give rise to skeletal damage due to low bone mineral density (osteoporosis) and fractures. Animal tests have shown that cadmium may be a risk factor for cardiovascular disease (Jarup, 2003).

For acute exposure, absorbed cadmium can cause symptoms such as salivation, difficulty in breathing, nausea, vomiting, a pain, anemia, kidney failure, and diarrhea. Inhalation of cadmium dust or smoke may cause dryness of the throat, headache, chest pain, coughing, increased uneasiness and bronchial complications (Lu et al., 2007).

2.2. MERCURY (NATURAL AND ANTHROPOGENIC SOURCES)

Mercury occurs naturally in the earth's crust. Although it may be found in air, water and soil, mercury is mostly present in the atmosphere as a gaseous element. Mercury's major natural source results from the degassing of the earth's crust, emissions from volcanoes and evaporation from natural bodies of water. Mining metals also causes indirect mercury discharges to the atmosphere. Due to its long lifetime of approximately of 1 year in the atmosphere, mercury's dispersion, transport and deposition in the environment will cause harmful effects on ecosystems and human health.

Mercury may be present in the environment in several forms: elemental or metallic mercury, inorganic mercury compounds and organic mercury compounds.

Pure mercury is a volatile liquid metal. It has traditionally been used in products like thermometers, switches, barometers and instruments for measuring blood pressure.

Mercury is naturally present in many rocks including coal. When coal is burned, mercury is released into the environment. For this reason, coal-burning power plants are one of the largest anthropogenic sources of mercury emissions to the air, in addition to all domestic human-caused mercury emissions. Burning hazardous wastes, producing chlorine, breaking mercury products, and spilling mercury, as well as the improper treatment and disposal of products or wastes containing mercury, can also contribute to its release into the environment (EPA, 2009).

Mercury compounds are produced in small quantities for chemical and pharmaceutical applications. In ancient Greece mercury was used as a cosmetic to lighten the skin (Jarup, 2003): in some sub-Saharan African countries the use of cosmetic products to bleach or to lighten the skin is still frequent. The long-term use of some pharmacologic compounds (hydroquinone, glucocorticoids and mercury) can cause severe health adverse effects (Jarup, 2003). Large quantities of mercury compounds are still used for amalgamation in illegal gold mining, in some developing countries.

Anthropogenic sources of mercury and their compounds may result basically from the same sources enunciated for Cd. In addition, underground mining, mining quarrying, opencast and, production of phytopharmaceutical products and biocides, pharmaceutical industry, landfills, urban waste treatment plants, industrial waste-water treatment plants, etc. (E-PRTR, 2010).

Exposure to mercury may mainly occur as a consequence of the deposition from air into water or into soil. By natural biological processes certain micro-organisms can change mercury into methyl mercury, a highly toxic and stable form that builds up in fish, shellfish and animals that eat fish, accumulating in the food chain. General population is exposed to methyl mercury through the food chain; fish and shellfish are the main source of exposure through the ingestion pathway (EPA, 2009).

Breathing mercury vapor is another possible exposure pathway. This can occur when elemental mercury or products that contain elemental mercury break and release mercury into air, in especial in indoor spaces without enough ventilation. Nevertheless, the main exposure pathway is through food chain and not by inhalation (EPA, 2009).

High level of mercury can cause brain damages, heart, kidneys and affect the immunologic system. Fish consumption does not constitute the main cause of health problems as mercury's quantity is not high. Nevertheless high levels of metylmercury in the bloodstream of little children may affect nervous system, affecting the normal thinking and learning (EPA, 2009)

Mercury's level in European countries has declined in the last few years, but is increasing in countries outside Europe, raising the level in the environment. Emissions are estimated to have declined mainly due to improved controls on mercury cells and their replacement and the generic fall in coal use (EPA, 2009).

2.3. LEAD (NATURAL AND ANTHROPOGENIC SOURCES)

Lead is a naturally occurring heavy metal often used in industry. It may be found in air, water, foodstuff, soil and dust either from natural sources, or from anthropogenic sources. In the past most of the lead emissions to the environment was due to petrol. This decreased in the last few years, in particular in developed countries, mostly due to the introduction of unleaded petrol. Lead can be found in several categories of use: batteries, petrol additives (no longer used in developed countries), rolled and extruded products, alloys and pigments. The most significant anthropogenic lead sources results basically from the same sources as for Cd and Hg (energy sector, production and processing of metals; mineral industry; chemical industry; waste and waste water management; paper and wood production processing and animal and vegetable products from the food and beverage sector (E-PRTR, 2010).

Lead's exposure can occur from both anthropogenic and natural sources: battery plants, glass industry, and occupational exposure for inorganic lead in mines and smelters and exposure pathways through air near point emissions like coal power plants (combustion). In the environment, lead bio-accumulates in most organisms and is toxic to plants, animals and micro-organisms (UNEP, 2008).

Airborne Pb may be deposited on soil, water and crops and also be transported through the raining containing this metal. Exposure can occur through food chain, drinking water, soil ingestion and dust inhalation. Up to 50% of inhaled inorganic Pb may be absorbed in the lungs. Adults take up 10–15% of Pb in food, while children may absorb up to 50% through the gastrointestinal tract. Lead elimination is slow and mainly by urine. It is also accumulated in the skeleton and is released from this compartment very slowly. Half-life of Pb in blood is about 1 month and in skeleton about 20–30 years (Jarup, 2003).

Acute exposure to lead's high levels may cause vomiting, diarrhea, convulsions, coma or even death. Chronic exposure, even to small amounts of lead, can be hazardous, especially to children under 6 years old who are particularly vulnerable to lead exposure; low levels of exposure to lead can cause neurodevelopment effects in children. Anemia, brain and nervous system damage are the health consequences of most concern. Other effects to generic exposed receptors include cardiovascular, renal, gastrointestinal, hematological and reproductive effects. In adults inorganic lead does not penetrate the blood-brain barrier but this barrier is less developed in children. A high gastrointestinal uptake and a permeable blood-brain barrier make children critical receptors to lead exposure and consequent severe brain damages (UNEP, 2008).

Organic lead is able to penetrate body and cell membranes, some forms of organic lead penetrate the skin easily and cross blood-brain barrier in adults and in this way adults can also suffer brain damage related to acute poisoning from organic lead compounds.

Lead in air comes mainly through industrial emissions, smelters and refineries. Dust and soil can be significant exposure pathways resulting either from deposition or from erosion of lead-bearing rocks. Dust may be also originated by lead-based paints or from outside soil. Water may be contaminated by deposition and lead can also enter into the water supply from lead solder in plumbing or lead pipes at homes. Foodstuff may be contaminated by atmospheric deposition of airborne lead or by irrigation with contaminated water (Jarup, 2003).

3. Heavy Metals European Emissions

A detailed air emission inventory is available at the European Pollutant Release and Transfer Register. This database provides easily accessible key environmental data from industrial facilities in 27 European Union Member States, including Iceland, Liechtenstein and Norway. The register contains annual data reported by some 24,000 industrial facilities covering 65 economic activities within the following nine industrial sectors: energy, production and processing of metals, mineral industry, chemical industry, waste and waste water management, paper and wood production and processing, intensive livestock production and aquaculture, animal and vegetable products from the food and beverage sector and other activities. Some information on releases from diffuse sources is also available, and intended to be gradually enhanced (E-PRTR, 2010).

For each facility, information is provided concerning the amounts of pollutant releases to air, water and land as well as off-site transfers of waste and of pollutants in waste water from a list of 91 key pollutants including heavy metals, pesticides, greenhouse gases and dioxins. Data provided in the register falls under seven classified groups: greenhouse gases, other gases, heavy metals, pesticides, chlorinated organic substances, other organic substances, inorganic substances (E-PRTR, 2010).

Releases and transfers from European member states must be reported according to the E-PRTR Regulation: EC Nº 166/2006 of the European Parliament Council Directives 91/689/EEC and 96/61/EC. The information to be reported annually by each facility for which the applicable thresholds are exceeded are the following: (i) Releases to air, water and land of any of the 91 E-PRTR pollutants; (ii) Off-site transfers of any of the 91 E-PRTR pollutants in waste water destined for waste-water treatment outside the facility; (iii) Off-site transfers of waste (reported as tones per year) for recovery or disposal.

The reported releases include any introduction of any of the listed pollutants into the environment as a result of any human activity whether deliberate, accidental, routine or non-routine, at the site of the facility. E-PRTR also contains information on releases from diffuse sources into water which will be upgraded and gradually extended (E-PRTR, 2010). The first report was released in 2009 containing data reporting to the year of 2007. The following report will be released in 2010 covering data from 2008. Registered data is intended to be updated on an annual basis; each report will cover emissions released two years before. As it happened in 2010, the data available in the register will be updated each year in April.

Data of environmental releases from the following countries was extracted: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Germany, Denmark, Estonia, Greece, Spain, Finland, France, Hungary, Ireland, Italy, Latvia, Luxembourg, Netherlands, Poland, Portugal, Romania, Sweden, Slovenia, Slovakia and United Kingdom. The extracted data refers to Cd, Hg, Pb releases into the air, water and soil and are summarized in the following tables. A few appreciations can be done considering environmental emissions reporting to industrial activity.

Production and processing of metals generated about 50% of the total European Cd and compounds emissions (18.66 t) (Table 1). This was followed by thermal power stations and other combustion installations sector (~20%) and mineral industry sector (~10%). Cd and compounds releases into water were mainly due to waste and waste water treatment (~63%) followed by production and processing metals industry (~13%) and paper and wood production processing sector (~6%). Waste and waste water treatment was the major responsible sector for emissions into soil (~72%).

TABLE 1. Cadmium and compounds emissions into the environment (extracted on March 2010).

Industrial activity (Cd emissions and compounds)	Facilities	Air	Water	Soil
Energy sector	142	5.72 t	986 kg	–
Mineral oil and gas refineries	24	992 kg	219 kg	–
Thermal power stations and other combustion facilities	117	3.72 t	743 kg	–
Coke ovens	1	1.01 t	2.41 kg	–
Production and processing metals	132	9.66 t	2.21 t	209 kg
Mineral industry	98	1.79 t	934 kg	–
Chemical industry	36	653 kg	991.1 kg	30.2 kg
Waste and waste water treatment	161	218 kg	10.9 t	1.20 t
Paper and wood production processing	64	509 kg	1.06 t	102 kg
Animal and vegetable products from the food and beverage sector	9	47.8 kg	85 kg	128 kg
Other activities	6	26.3 kg	53.4 kg	–
Total	648	18.66 t	17.2 t	1.67 t

Mercury and compounds air emissions were in majority generated in the energy sector (~55%), in particular from thermal power plants and combustion facilities (~51%) (Table 2). This was followed by chemical industry sector (~17%) and production and processing metals industry (~13%). Energy sector (mineral oil and gas refineries industry) was also the major responsible for mercury and mercury compounds releases into water (~44%), followed by waste and waste water treatment industry sector (~32%); this last industry sector was also the great contributor to soil emissions (~79%).

Production and processing metals generated the majority of lead and lead compounds air emissions (~72%). This was followed by the energy sector (~15%), in particular from thermal power stations and other combustion installations (~11%). Mineral industry generated about 11% of total air emissions. Waste and waste water treatment sector was the major responsible for lead and compounds releases into water (~57%) and into soil (~66%). Mineral industry sector contribute by about 16% to water emissions and production and processing metals sector was responsible with about 26% lead and compounds emissions to soil.

TABLE 2. Mercury and compounds emissions into the environment (extracted on March 2010).

Industrial activity (Hg emissions and compounds)	Facilities	Air	Water	Soil
Energy sector	249	19.8 t	4.65 t	2.7 kg
Mineral oil and gas refineries	28	940 kg	125 kg	–
Thermal power stations and other combustion facilities	216	18.5 t	4.52 kg	2.7 kg
Coke ovens	2	250 kg	2.29 kg	–
Manufacture of coal products and solid smokeless fuel	3	85.4 kg	–	–
Production and processing metals	91	4.77 t	456 kg	9.49 kg
Mineral industry	114	4.00 t	113 kg	–
Chemical industry	78	6.14 t	1.85 t	–
Waste and waste water treatment	284	1.22 t	3.32 t	232 kg
Paper and wood production processing	42	199 kg	1.01 t	27.3 kg
Animal and vegetable products from (food and beverage sector)	4	–	3.58 kg	23.3 kg
Other activities	4	13.0 kg	4.35 kg	–
Total	866	36.1 t	10.5 t	295 kg

TABLE 3. Lead and compounds emissions into the environment (extracted on March 2010).

Industrial activity (Pb emissions and compounds)	Facilities	Air	Water	Soil
Energy sector	85	80.9 t	21.6 t	–
Mineral oil and gas refineries	14	2.14 t	3.57 t	–
Gasification and liquefaction	1	232 kg	28.0 kg	–
Thermal power stations and other combustion facilities	68	61.1 t	18.0 t	–
Coke ovens	2	17.4 t	–	
Production and processing metals	185	398.3 t	22.2 t	18.8 t
Mineral industry	86	60.9 t	34.8 t	–
Chemical industry	51	2.34 t	9.61 t	749 kg
Waste and waste water treatment	364	5.41 t	124 t	44.7 t
Paper and wood production processing	59	3.17 t	3.74 t	1.98 t
Animal and vegetable products (food and beverage sector)	10	–	190 kg	1.93 t
Other activities	6	–	421 kg	–
Total	845	551.74 t	217 t	68.2 t

In what concerns to country's emissions data was also extracted and is presented in the Table 4. The inventory data refers to those three countries that generated higher heavy metals emissions (Cd, Hg and Pb) either to air, water and soil.

TABLE 4. Cadmium, Mercury and Lead, and compounds air emissions per country.

Cd and compounds					
Air		Water		Soil	
France	2.96 t	Spain	3.98 t	France	260.64 kg
Spain	2.94 t	Poland	3.28 t	Germany	214.00 kg
Czech Republic	2.76 t	Italy	2.35 t	U.K.	209.00 kg
Hg and compounds					
Germany	7.64 t	Italy	4.18 t	France	104.63 kg
Spain	4.25 t	Poland	2.61 t	Germany	32.90 kg
Poland	3.84 t	Spain	1.01 t	U.K.	18.80 kg
Pb and compounds					
Germany	68.20 t	Poland	99.34 t	U.K.	19.61 t
France	61.86 t	U.K.	32.87 t	France	10.31 t
Belgium	55.70 t	Spain	19.02 t	Germany	8.52 t

The highest cadmium and compounds air emissions was reported by France and Spain (~16% each), followed by Czech Republic (~15%). Spain generated the highest emissions into water (~22%) while France was the highest contributor to soil emissions (~36%).

Germany was responsible to the highest mercury and mercury compounds air emissions (~21%) followed by Spain (~12%) and Poland (~10%). Italy generated the highest mercury and compounds emissions into water (~39%) and France was responsible for the highest slice emissions into soil (~66%).

The highest lead and compounds air emissions were reported by Germany (~13%), followed by France (~11%) and Belgium (~10%). Poland was by far the highest contributor to water emissions (~44%) and U.K. was responsible for the highest soil emissions (~51%).

4. Heavy Metals Emissions into the Environment in Portugal

Data for the Portuguese inventory emissions was also extracted from the E-PRTR database. The following table gives an overview of the industrial sectors emissions to air, water and soil.

Cadmium and cadmium compounds emissions were generated in majority from the paper and wood production processing (~30%) and from energy sector (~33%); in particular, mineral oil and gas refineries are responsible for about

75% of air emissions. Waste and waste water treatment was responsible for about 73% of water emissions and the only register to soil emissions comes from animal and vegetable products (food and beverage sector) industrial sector (Table 5).

TABLE 5. Cadmium and compounds air emissions in Portugal (extracted on March 2010).

Industrial activity (Cd emissions and compounds)	Facilities	Air	Water	Soil
Energy sector	6	325 kg	–	–
Mineral oil and gas refineries	2	245 kg	–	–
Thermal power stations and other combustion facilities	4	80 kg	–	–
Production and processing metals	4	280 kg	15.6 kg	
Mineral industry	3	78 kg	–	–
Chemical industry	1	–	94.3 kg	–
Waste and waste water treatment	3	11 kg	536 kg	–
Paper and wood production processing	7	364 kg	6.4 0 kg	–
Animal and vegetable products (food and beverage sector)	3	27 kg	62.7 kg	31.5 kg
Other activities	2	13 kg	15.3 kg	
Total	29	1.10 t	730 kg	31.5 kg

Energy was the major responsible sector for mercury and mercury compounds air emissions representing ~67% from thermal power plant and other combustion facilities and ~21% from mineral oil and gas refineries. Water and waste water treatment was the main contributor to water emissions (98%). There is no register to mercury and mercury compounds soil emissions (Table 6).

TABLE 6. Mercury and compounds air emissions in Portugal (extracted on March 2010).

Industrial activity (Hg emissions and compounds)	Facilities	Air	Water	Soil
Energy sector	4	432 kg	–	–
Mineral oil and gas refineries	1	138 kg	–	–
Thermal power stations and other combustion facilities	3	294 kg	–	–
Production and processing metals	1	87 kg	–	–
Mineral industry	2	39 kg	–	–
Chemical industry	1		1.8 kg	–
Waste and waste water treatment	3		111 kg	–
Paper and wood production processing	4	73 kg		–
Other activities	1	13 kg	15.3 kg	–
Total	16	644 kg	113 kg	–

Lead and lead compounds emissions were generated in majority from the production and processing metals (~66%) (Table 7). Energy sector and mineral industry were the following contributors representing 17% and 14% respectively of total 2007 air emissions. Waste and wastewater treatment sector was the main responsible for water emissions (~78%) followed by production and processing metals sector (~14%). There is no register for lead and lead compounds soil emissions.

TABLE 7. Lead and compounds air emissions in Portugal (extracted on March 2010).

Industrial activity - (Pb emissions and compounds)	Facilities	Air	Water	Soil
Energy sector	2	1.27 t	–	–
Mineral oil and gas refineries	1	500 kg	–	–
Thermal power stations and other combustion facilities	1	774 kg	–	–
Production and processing metals	4	5 t	288 kg	–
Mineral industry	3	1.06 t	29.1 kg	–
Chemical industry	–	–	–	–
Waste and waste water treatment	3	–	1.61 t	–
Paper and wood production processing	3	250 kg	62.6 kg	–
Animal and vegetable products (food and beverage sector)	1	–	80.4 kg	–
Total	16	7.59 t	2.07 t	–

5. General Overview of Methodologies Used on Exposure Assessment

Exposure assessment relies upon fields of statistics, biochemical toxicology, animal toxicology, atmospheric sciences, analytical chemistry, food sciences, physiology, environmental modeling and others.

The exposure is quantified based on the observed dose-response relationship between the receptor and the contaminant and the assessment includes a description of intensity, frequency and duration of exposure through different exposure compartments, routes of exposure, identification of potential receptors exposed and a prevision of how these factors might change with time and as a consequence of some actions or events. This also requires using monitoring data, exposure modelling techniques and mapping models. Exposure is generally quantified in terms of the predicted environmental concentration, which is calculated on both local and regional spatial scales from monitoring data, when available, or by using realistic worst-case scenarios. If this information is not available, the estimative may result from exposure models. It is necessary to produce a complete description of how, when, and where the exposure occurs or has occurred, by evaluating the sources as well as the releases, the extent and pattern of contaminant contact with humans or ecologically relevant biota (Dinis and Fiúza, 2009).

Most of the times many different sources are present as well as multi-pathways, and multiple chemicals exposure at the same time. The available assessment guidelines are based on an unrealistic assumption that the releases of chemicals, as well as their effects, are separated from each other; usually the normal procedure is to perform an exposure assessment by calculating the dose resulting for each one of the chemicals.

On the other hand, heavy metals uptake, absorption and subsequent metabolism are determined by chemical speciation of metals and this contributes to the intensity of their toxicity. Also the potential metal toxicity compound depends on the oxidation state of the metal and the solubility of the compound. The chemical speciation of metals to which humans are exposed in ambient air, drinking water or diet is not considered in dose assessment due to the lack of information (Paustenbach, 2000).

Many times specific health effects are associated with a specific exposure route (for instance, lung cancer is related with inhalation), however, most health effects associated with heavy metals exposure affect the whole body and not just a specific route of exposure, although it may influence the systemic dose (Paustenbach, 2000).

The absorption of different metals depends both on the form in which metals are present in the exposure medium and also the route of exposure. Very little investigation of combined metals exposure have been done; it appears that there is, at present, no practical alternative to treating the health impacts of different metals as being additive.

6. Exposure Assessment Methodology Applied to a Hypothetical Case Study

This section describes the methodology applied to assess the potential average daily dose and the lifetime average dose resulting from exposure to chemicals released from an industrial facility during a certain period of time. This approach uses current measures of heavy metals in the environment media where the receptors live, causing exposition during their daily activities.

Air emissions from thermal power plants and other combustion installations were considered to apply the exposure assessment methodology (the main contamination from these facilities comes from air emissions). Coal contains many trace elements that are released during combustion to the surrounding environment. Some of the trace elements present in coal are heavy metals, such as nickel, chromium and those of interest in this study: cadmium, mercury and lead.

Air emissions will generate multipathways exposure assessment which extends the evaluation of the direct inhalation of contaminants, derived from air dispersion and deposition, to consider a variety of indirect pathways that include the deposition of contaminants to soil, water and vegetation, and the subsequent transfer and accumulation in the food-chain. Dermal absorption can also be an exposure pathway from direct contact with contaminated water and soil.

It is well known that the health effects and environmental impacts arising from coal thermal power stations and other combustion installations are originated by the mobilization of toxic elements from ash. The mobilization of these elements into the environment depends on various factors like climate, types of soils, indigenous vegetation and agricultural practices. In this way, the predicted exposure to contaminants resulting from a facility's emissions depends not only upon the levels of the emissions but also on the characteristics of the local land and its uses by the local population. A subsistence farmers (adults and children) scenario was considered for applying the methodology (Zemba et al., 2002).

Although both children and adults may be exposed to the same pathways, children's rates of exposure to compounds (as expressed per unit body weight) are frequently higher and they may be subject to different types of health risks. Generally children younger than the age of 6 are considered critical receptors as they may easily get exposed by the ingestion of contaminated soil. Infants and children are critical receptors either due to their critical stages of physical and mental development either due to the fact that they receive higher pollutant dose compared to adults as they have lower body weight and higher breathing rate (consume more food per kilogram of body weight than adults). Also, children's developing organs and tissues are more vulnerable to the toxic effects of certain chemicals. With greater exposure and more severe health effects, chemicals in food are more harmful to children than adults. In general, children living in the vicinity of source emissions have the highest health risks. Adults are also at risk mostly from contaminated water and from inhaling dust from source emissions.

For cadmium exposure the health effects on children's are expected to be similar to the effects seen in adults (kidney, lung, and bone damage depending on the route of exposure). However, results from animal studies showed that younger animals absorb more cadmium than adults; younger animals are more predisposed than adults to a loss of bone and decreased bone strength from exposure to cadmium; babies of animals exposed to high levels of cadmium during pregnancy had changes in behavior and learning ability and high enough exposures to cadmium before birth can reduce body weight and affect the skeleton (ATSDR, 2008).

For the farmer subsidence scenario, air, soil, food crops (leafy vegetables); pasture and animal products (meat and milk) were considered as the representative exposure medium. The potential exposure routes were identified: inhalation (dust and soil resuspension from contaminated soil), ingestion (soil ingestion, leafy vegetables, meat and milk ingestion) as represented in Figure 1.

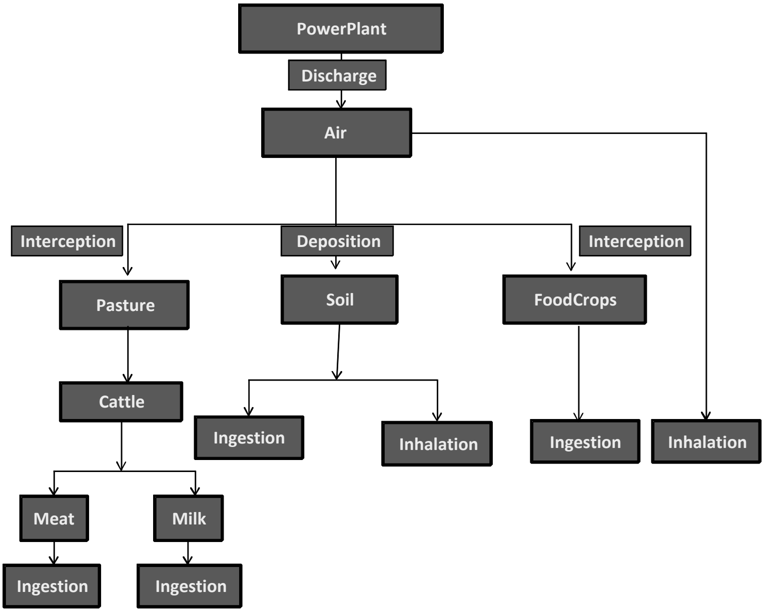

Figure 1. Main exposure pathways considered in the subsidence farmer scenario.

In order to perform dose calculations and exposure assessment the concentration of each chemical/heavy-metal needs to be measured or estimated in air, soil, food crops (leafy vegetables) and animal products (meat and milk). Also a wide range of fate and transport parameters are necessary to conduct the assessment as well as exposure parameters are needed to characterize the scenario. Different default values for exposure parameteres should be considered for children and adults as described in U.S. EPA, Exposure Factors Handbook (EPA, 1997a).

6.1. EXPOSURE ASSESSMENT – DOSE CALCULATIONS

Cadmium is classified as a possible human carcinogenic, in particular due to inhalation (IRIS, 1991). Lead is also classified as probable human carcinogenic, although quantifying lead’s cancer risk involves many uncertainties: age, health, nutritional state, body burden, and exposure duration influence the absorption, release, and excretion of lead. The U.S. Carcinogen Assessment Group recommends that a numerical estimate should not be used to lead (IRIS, 2004). Mercury (elemental) is not classifiable as human carcinogenic based, however, on inadequate human and animal data (IRIS, 1995).

Cadmium and lead are volatilized on combustion and preferentially get adsorbed on the fly ash. Mercury remains almost entirely in the volatilized state and tends to escape to the atmosphere as vapors. However, mercury species that dominate deposition to soil and surface water are oxidized forms associated with particulate matter. In these conditions mercury deposition rates can be relevant near the source and can effectively influence the amount of mercury that contaminate soil, vegetation and surface waters and ultimately bio-accumulates in local fish populations. The U.S. EPA (1997b) has published fate-and-transport algorithms for predicting the deposition of mercury released from combustion sources.

The herein example focus on the methodology applied to the assessment of the exposure to cadmium emissions in a particle state matter; atmospheric cadmium produced by combustion processes occurs in the form of particulate matter at very small particles sizes. Cadmium oxide is the principal chemical species in the air, nevertheless some cadmium salts (e.g. sodium chloride) can escape to atmosphere, especially during incineration. Both forms are stable and won't suffer significant chemical transformations.

The exposure may occur directly by the inhalation of the cadmium attached to particles and indirectly by their ingestion within food (milk, meat, leafy vegetables and incidental ingestion soil). Drinking water may not be considered if we assume that all ingested water comes from a well that is not contaminated. The calculation of exposure will depend on (1) contaminant concentration in the media relevant to the exposure scenario being considered; (2) the rate at which individual consumes (ingests or inhales) the given medium; (3) the frequency and duration of exposure; (4) the individual's weight. Exposure can be expressed in a generic expression as (Zemba et al., 2002):

$$\mathrm{I}\left(\frac{\mathrm{mg}}{\mathrm{kg.day}}\right)=\frac{\mathrm{C}\cdot\mathrm{CR}\cdot\mathrm{EF}\cdot\mathrm{ED}}{\mathrm{BW}\cdot\mathrm{AT}} \qquad (1)$$

where I is the intake (amount of contaminant, $\mathrm{mg.kg^{-1}.day^{-1}}$); for evaluating exposure to noncarcinogenic contaminants, the intake is refered to as average daily dose, ADD; for evaluating exposure to carcinogenic contaminants the intake is referred to as lifetime average daily dose, LAAD; C is the concentration in media of concern (mg/kg for soil or $\mathrm{mg/m^3}$ for surface water); CR is the consumption rate (kg/day for soil or $\mathrm{m^3}$/day for water); EF is the exposure frequency (days/year); ED is the exposure duration (years); BW is the body weight (kg) and AT is the averaging time equal to 25,550 days based on a lifetime exposure of 70 years; for noncarcinogens, averaging equals ED (years) multiplied by 365 days per year. The indirect exposure can be calculated by (Zemba et al., 2005):

$$I_{ind}\left(\frac{mg}{kg \cdot day}\right) = I_{soil} + I_{lv} + I_{milk} + I_{meat} \qquad (2)$$

where I_{ind} is the total daily intake of the contaminant by indirect pathways (mg.kg^{-1}.day^{-1}); I_{soil}, I_{lv}, I_{milk} and I_{meat} are the daily intake of contaminant from soil, leafy vegetables, milk and meat (mg.kg^{-1}.day^{-1}), respectively. The intake of contaminant due to incidental ingestion of soil is calculated by (Zemba et al., 2002):

$$I_{soil}\left(\frac{mg}{kg \cdot day}\right) = \frac{CS \cdot IR \cdot FI}{BW} \qquad (3)$$

where CS is the average soil concentration over exposure duration (mg.kg^{-1}); IR is the consumption rate of soil (kg/day); FI is the fraction of ingested soil that is contaminated (unitless). The intake of contaminant due to the ingestion of leafy vegetables is calculated by (Zemba et al., 2002):

$$I_{lv}\left(\frac{mg}{kg \cdot day}\right) = \frac{CV \cdot IR \cdot FI}{BW} \qquad (4)$$

where CV is the average leafy vegetables concentration over exposure duration (mg.kg^{-1}); IR is the consumption rate of leafy vegetables (kg/day); FI is the fraction of ingested leafy vegetables that is contaminated (unit less). The intake of contaminant due to the ingestion of milk and meat is calculated by (Zemba et al., 2002):

$$I_{lv}\left(\frac{mg}{kg \cdot day}\right) = \frac{CM \cdot IR \cdot FI}{BW} \qquad (5)$$

where CM is the average animal tissue or product (meat or milk) concentration over exposure duration (mg.kg^{-1}); IR is the consumption rate animal tissue or product (meat or milk) (kg/day), FI is the fraction of ingested animal tissue or product (meat or milk) that is contaminated (unitless). The intake of contaminant due to the inhalation is calculated by (Zemba et al., 2002):

$$ADI\left(\frac{mg}{kg \cdot day}\right) = \frac{CA \cdot IR \cdot ET \cdot EF \cdot ED \cdot 0{,}001}{BW \cdot AT \cdot 365} \qquad (6)$$

where ADI is the average daily intake of the contaminant via direct inhalation (mg/kg^{-1}.day^{-1}); CA is the total air concentration over exposure duration (μg/m^3);

IR is the inhalation rate (m^3/h); ET is the exposure time (h/day); EF is the exposure frequency (day/h); 0.001 is the units conversion factor (μg/mg) and conversion factor (days/year).

After estimating the average daily intake the next step is the calculation of excess the lifetime cancer risks and non-cancer health hazards resulting from the exposure scenario considered.

Carcinogenic risks are calculated as the product of long-term average dose and the carcinogenic power factor (also known as slope factor). Carcinogenic risks are a conservative estimate of the incremental probability that an individual will develop cancer as a result of a specific exposure to a carcinogenic compound; the estimative is done by multiplying an individual's lifetime average daily dose (LADD) of a compound by the compound's unit risk factor or cancer slope factor (CSF) (IRIS, 2009). An individual's overall cancer risk is the sum of the cancer risks from all the compounds of concern.

The acceptable risk is generally defined as 10^{-6} for the general public and 10^{-5} for occupational works. This means that an additional one case of cancer is accepted for populations of 1 million or 100,000, respectively. A risk level of 1 in a million, or 1 in one hundred thousand, also implies a likelihood that up to one person out of one million (or 100,000) equally exposed people would contract cancer if exposed continuously (24 h day^{-1}) to a specific radiation dose over 70 years (an assumed average lifetime).

Non-carcinogenic risks are assumed to exhibit a threshold below which no adverse effects are expected to be observed. As such, non-carcinogenic health hazards are evaluated by comparing an individual's exposure to a compound against a reference dose RfD for oral exposures. Reference concentrations and reference doses are used to assess the likehood of chronic toxic health effects from inhalation and oral exposure, respectively. The risk is calculated as a hazard quotient ratio (HQ) of the average daily dose (ADD) to a reference dose response (RfD) both in the units of $mg.kg^{-1}.day^{-1}$.

A total hazard ratio is obtained as the sum of all hazard ratios calculated for individual exposure routes and compounds (Hazard Index - HI) for a particular exposure pathway. The calculated HI value is compared to a benchmark; a HI value of 1.0 or less than 1.0 indicates that no adverse human health effects (noncancer) are expected to occur although values less than 1.0 may not be risk free (IRIS, 2009).

Data to evaluate the chronic toxic effects of Cd may be obtained from the Integrated Risk Information System (IRIS): RfD (Cd in food) = 0.001 $mg.kg^{-1}.day^{-1}$ (IRIS, 1991).

The quantitative risk estimate from inhalation exposure may be presented by a unit risk, being the quantitative estimate in terms of risk per mg/m^3: Cd = 1.8 $(mg/m^3)^{-1}$ or by a slope factor, being the result of the aplication of low dose extrapolation prodecure presented as the risk per $mg.kg^{-1}.day^{-1}$: Cd = 6.10 $(mg/m^3)^{-1}$ (IRIS, 1991).

7. Measures to Reduce or to Mutigate Exposure to Heavy Metals

In order to reduce or to mitigate exposure to heavy metals, risk analysis should consider not only the direct emissions to the atmosphere but also the impacts of heavy metals deposition, transport and transfer in the food chain and in the drinking water. This is why a complete exposure assessment and a consequent risk assessment are needed. Also a cost-benefit analysis for measures to be taken should be addressed based on the impacts resulting from the exposure (ESPREME, 2004).

At individual level appropriate measures in case of heavy metals exposure include: (i) limit the exposure; (ii) decrease absorption with dietary suggestions may helpful to inhibit intake and uptake of cadmium, mercury and lead. For instance, Fe reduces uptake of Cd and Pb; Ca reduces the uptake of Cd; high sulfur foods such onions and garlic probably help the body blocking uptake and decreasing retention of many toxic metals, thus decreasing the release of stored metals and protecting the vulnerable tissues (VMICC, 2003). General population can contribute to heavy metals reduction efforts by purchasing metals free products (for example, Hg free); correctly dispose products that contain heavy metals and reducing demand for products whose production leads to the release of heavy metals into the environment (EPA, 2009).

A European database collected suggested methods and options for reducing the metals occurrence in Europe from industrial facilities (ESPREME, 2004). The database contains data for every European country. Experts from about twelve countries were involved in the process of the data collection (ESPREME, 2004).

The general Access database contains 483 files for each European country; those refer the processes responsible for emissions to the air, the measures taken for controlling these emissions, the investment and operating costs for each process, the unabated emission factors generated from individual process and the implementation of specific abatement methods.

A short list of several measures for decreasing the emissions originated in industries was extracted. They are classified as primary level and secondary level. Primary measures are considered for emissions from combustion plants (fuel combustion for energy production); fuel supply measures and combustion technology improvements. Primary measures include technical and non-technical actions: (i) the first group concerns the minimization of the heavy metals content in fuels and raw materials, as well as technology and fuel switching towards methods generating lower or zero HM emissions and technologic modifications; (ii) non-technical actions implies higher surveillance procedures, reducing consumption of heavy metals contain/releasing products and good housekeeping of environment of heavy metals releasing processes (ESPREME, 2004).

Generic secondary measures include (i) particulate control techniques (electrostatic precipitators, filters, cyclones, wet scrubbers); (ii) absorption techniques to achieve heavy metals emissions reduction, NO_x reduction techniques (aiming the Hg reduction); (iii) adsorption methods (activated carbon, impregnated sorbents, etc.) (ESPREME, 2004).

8. Conclusions

Concerning heavy metals sources and emissions into the environment, some statements may be pointed out. In the last few years, the biggest reduction in emissions from European countries has come from industrial processes. Lead and cadmium emissions have both decreased from certain industrial sectors, such as metal refining and smelting activities. This reflects an improved emissions control and, for some countries, the result of economic restructuring and the closure of older and more polluting industrial facilities (EEA, 2010). Since 1990 the largest mercury reduction (in absolute terms) has been achieved by the energy sector (electric power and heat generation) closely linked to less use of coal, which contains mercury as contaminant. Also, the fuel replacement in many countries from coal to gas and other energy sources, the closure of older inefficient coal-burning plants and the improvement of the processing equipment are responsible for the past decreases in emissions from this sector (EEA, 2010).

For the heavy metals considered of great concern in this study (Cd, Hg and Pb), the main emissions sources in 2007 were from energy-related sources associated with fuel combustion: production of electric energy and heat generating facilities and also from combustion in industrial facilities. Lead emissions from the road transportation sector were greatly reduced since 1990. However, this activity may continue to be an important source of lead: the residual lead in fuel, from engine lubricants and parts, and from tyre and brake wear contribute to the ongoing lead emissions from this sector (EEA, 2010).

The coal combustion is still the major source for the heavy metals focused in this study, except for lead. In general, big power plants are becoming more and more efficiently controlled resulting in developed effort to reduce emissions by mainly changing and improving their technology. Nevertheless, emissions from residential units are still being largely uncontrolled. Waste disposal is under-estimated as a source of heavy metals releases to the atmosphere. Alternative renewable energy sources will contribute to reduce emissions from combustion of coal (ESPREME, 2004).

The continuous reduction on heavy metal emissions in Europe for the last 40 years is due mainly to a better knowledge of heavy metals sources, emissions, pathways, transport and fate in the environment. Further progresses have been made in assessing sources and emissions to environment in Europe, at national and regional level. Also, the emission control with more reliable equipment has resulted in more efficient regulatory efforts to reduce heavy metal emissions from anthropogenic sources and it is clear that the most effective way to reduce or to eliminate the exposure to heavy metals in the environment is taking actions at the sources level (EEA, 2010).

Finally, to evaluate the exposure of heavy metals in the environment the procedure still consists in studying isolated contaminants. This means that we still lack adequate data, methods and models to assess multiple exposures and consequently to evaluate the risk to which receptors are exposed (Hinton and Aizawa, 2007).

In 1996 the U.S. EPA started to include mixture of chemicals in its risk assessment for pesticides that have the same mode of action; however, this limitation has again the same additive effect for mixtures (Hinton and Aizawa, 2007). The recent EPA guidance for mixtures for human health risk characterization does not suggest any single approach; instead it provides a number of options for consideration (Hinton and Aizawa, 2007). The ATSDR presented a series of "Interaction Profiles" to characterize the mixtures effects. Due to the lack of data, ATSDR applies an additional safety factor of 10 for mixtures of non-cancerous chemicals and 100 for cancerous chemicals mixtures (Hinton and Aizawa, 2007).

Many agencies have been involved in chemical mixtures exposure assessment (U.S. EPA, NIEHS, ASTDR, U.S. DHHS) and they all recognize the need of substantial research for risk assessment methods applicable to mixtures of heavy metals. In the short term, hazard indices calculations and additive carcinogenic risk calculation is likely to remain the predominant form of assessment for mixtures. A strategic approach involving a multinational, multidisciplinary strategic research methodology is urgently needed. The dispersion of many heavy metals beyond national boundaries implies that solutions must result from a long-term development plan of global research. Collaborative funding calls and joint proposals among several countries will surely allow a more rapid and efficient research on this difficult issue (Hinton and Aizawa, 2007).

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HEAVY METALS ACTIVITY MECHANISMS AT CELLULAR LEVEL AND POSSIBLE ACTION ON CHILDRENS BODIES

GHEORGHE COMAN* AND CAMELIA DRAGHICI
Transilvania University of Brasov
29 Eroilor Bldv., 500036 Brasov, Romania

Abstract. Different natural and artificial sources of pollution (manufacturing, metallurgy, mining, constructions, and incinerators) can contaminate the environment with heavy metals. If natural sources contribute to environmental pollution only to a little extent, artificial ones, which result from different human activities, contribute decisively to the pollution of the environment. Recently, interest in heavy metals (Pb, Hg, Cu, Zn, Cd, Sn, Ni, Cr, Mn, Se, As) has increased as they contaminate the environment and severely affect human and animal health. This paper presents some theoretical toxicity aspects of three elements (cadmium, lead, arsenic) and possible additive or synergistic action in human bodies, especially in children. Evaluating these actions is essential for risk assessment and management of heavy metals as single or mixture environment pollutants.

Keywords: Heavy metals, metallothioneins, cadmium, lead, arsenic, toxicity

1. Introduction

Large scale production of a variety of chemicals, energy production as well as other development activities (agriculture, transport, urbanization, health, tourism and services), have led to the release of huge quantities of waste into the environment. This waste may cause contamination of air, surface water, ground water, soil, sediments and living organisms.

The pollutants are transported to different distances, depending on the geographic area, its geology and meteorological factors (wind, rain, fog, thermal inversions) and trophic or feeding level. A substantial amount of this waste is potentially hazardous to the environment and is dangerous to living organisms (Hegazy, 2008; Coman et al., 2008; Dobra et al., 2006).

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* To whom correspondence should be addressed: gh.coman@unitbv.ro

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Some heavy metals such as Cu, Zn, Mn, Se, are essential for normal animal and plant growth and development, since they are constituents of many co-enzymes, enzymes and other functional proteins. Elevated concentrations of these metals in the environment can lead to toxicity symptoms and inhibition of growth and development of most living organisms (Hall, 2002).

Heavy metals are metals having atomic weight higher than 40.1 (exceeding that of calcium) and the soluble forms are generally ions or unionized organo-metallic chelates or complexes (Henry, 2000).

The large concentrations of heavy metals in soil inhibit microbial activity by:

- altering enzyme conformations;
- blocking essential functional groups (sulfhydryl, amine, hydroxyl, carboxyl, imidazolyl);
- displacing other essential metal ions (deficiency effects).

Human and animal health is aggravated by the persistence of heavy metals in environment, therefore heavy metal contamination represents one of the most pressing threats to water and soil resources as well as human and animal health (Impelliteri and Saxe, 2003).

The solubility of heavy metals in soil, water and body fluids is predominantly controlled by pH and the presence of other compounds. In living organisms, citric acid, malic acid, cysteine, histidine, and some peptides and polypeptides (phytochelatins, metallothioneins) are potential high affinity ligands for heavy metals, and could play a role in their transport, storage, activity, effects, tolerance and detoxification (Coman et al., 2006; Clemens, 2001).

Metallothioneins are small soluble metal-binding polypeptides (6,000 Da) that contain more sulfhydryl(thiol) groups than other polypeptides. These thiol groups provide the basis for high-affinity binding of several heavy metals: mercury, zinc, copper, cadmium, lead (Pages et al., 2008).

Ferritin is an iron storage protein that may serve as a zinc and cadmium detoxifying agent, because it also has affinity for these metals (Coman et al., 2003).

Transferrin is a ferric iron transport protein and that can also transport manganese in plasma or across cell membranes.

Ceruloplasmin is a copper transporting protein that is also involved in converting ferrous iron to ferric iron, which then binds to transferrin (Leslie et al., 2006; Cousins et al., 2006).

The anthropogenic contribution to the levels of the heavy metals in soil, water, air, food and living organisms is well recognized, since metals are one of the oldest toxicant known to humans, probably prior to 2000BC, due to their very early use (Klaassen, 2008).

The first description of abdominal colic is credited to Hippocrates in 370BC, who describes these symptoms in a man who worked in lead mining.

Heavy metals are redistributed in the environment by biologic and geologic processes (Bradl, 2005). As byproducts of combustion they are adsorbed on the particulate matter, transported in the atmosphere, then deposited by rain on soil and biota. Rain water dissolves and transports heavy metals to underground waters, rivers and the ocean, where they can precipitate and then deposit as sediments.

Incorporation of heavy metals into food cycles of plants and animals (biomagnification) leads to moving metals in the environment, and it contributes to the distribution and redistribution of metals (ecotoxicological effects).

Human exposure to heavy metals (dietary, occupational, environmental) leads to development of health effects: diabetes, hypertension, cancer, cardiovascular, renal, and neurological diseases. Children are much more vulnerable than adults to heavy metals exposure because of their more rapid growth rate and metabolism. The developing fetus is highly susceptible to heavy metals through maternal blood.

The health effects of exposure to heavy metals on children's bodies may be: retardation and brain damage, cardiologic and hematological diseases, liver and gastrointestinal diseases, kidney and urinary tract diseases, neurological diseases, chemical allergy, genotoxicity, mutagenesis and carcinogenesis. High levels of exposure may even be fatal.

2. Cadmium Exposure and Its Effects

Introduction of cadmium into the human body occurs by ingestion of contaminated food and water, by inhalation of industrial dust and fumes, as well as by inhalation of cigarette smoke (Figure 1). The ingested cadmium passes through the gastrointestinal tract (GI tract), and most of it is eliminated in the feces. The inhaled cadmium arrives in the lungs, enters the bloodstream and is finally released by urine.

In the bloodstream cadmium circulates in erythrocytes. In the liver it induces biosynthesis and is bound to metallothioneins or other low molecular weight polypeptides that exert a protective tissues effect from cadmium toxicity (Coman and Draghici, 2004). In the kidney the metallothionein-bound cadmium, released from the liver, is filtered in the glomeruli and then reabsorbed by the proximal tubules. Here the complex is degraded, releasing high levels of free cadmium locally which accumulate in the kidney. Inorganic cadmium can be taken up into the kidney cell from the basolateral membrane, and is more toxic to it than cadmium metallothionein complexes (Zalplus and Ahmad, 2003).

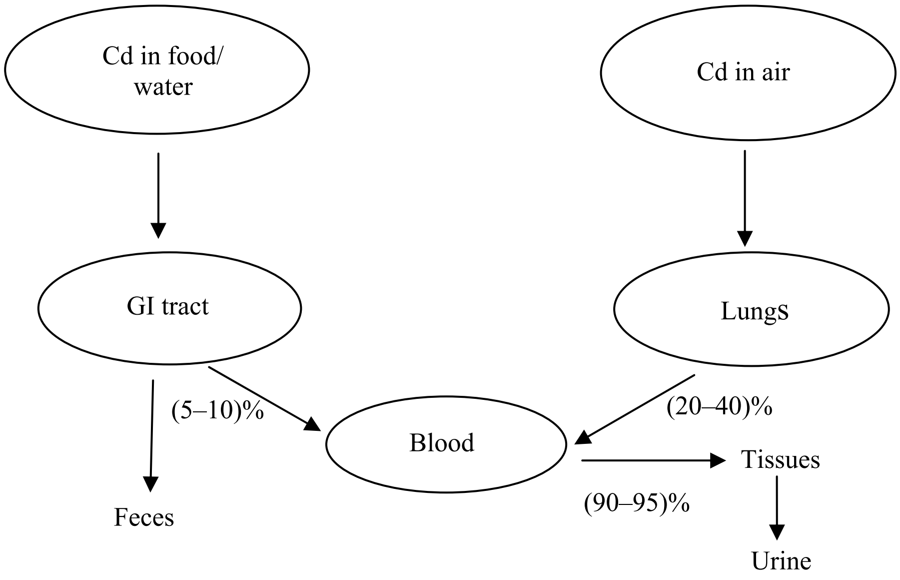

Figure 1. Cadmium metabolic circuit in humans.

Inorganic cadmium is toxic to tubular renal cells and glomeruli, initially causing tubular cell necrosis and degeneration, later progressing to interstitial inflammation and fibrosis. The kidney is the main target organ of cadmium toxicity. Cadmium induced renal toxicity is reflected by proteinuria, as a result of renal tubular dysfunctions. Inorganic cadmium can bind other low molecular proteins or other macromolecular complexes for renal uptake, which can contribute to cadmium nephropathy.

The first red flag signalling that cadmium renal burden is reaching threshold values is the presence of large quantities of low molecular weight proteins in the urine (β-2-microglobulins, metallothionein, glucozaminidase, α_1-microglobulin, lysozyme, immunoglobulin light chain and retinol-binding protein), due to the impairment of the reabsorbtion process (Chen et al., 2006).

By binding with metallothioneins, cadmium's mobility through diffusion is highly reduced and the metal is therefore prevented from binding to enzymes or other functional proteins (Figure 2).

At high levels of exposure, massive proteinuria is registered, as well as cortical necrosis associated with increased levels of uric acid, amino acids, glucose, calcium, and phosphates in the urine. The loss of calcium and phosphates produces a dysfunction in calcium metabolism, ultimately causing osteoporosis, osteomalacia, nephrolithiasis, and nephrocalcinosis.

The presence of larger proteins (albumin and transferrin) in the urine, after occupational cadmium exposure, suggests glomerular damage as well.

In addition to renal injury, cadmium chronic toxicity may play a role in the lung and prostate cancer development because it stimulates cell proliferation and inhibits DNA repair (Waalkes, 2000).

Cadmium is a highly cumulative poison with a biologic half-life estimated at about 20–30 years in humans. It's accumulation with the age is presented in Figure 3.

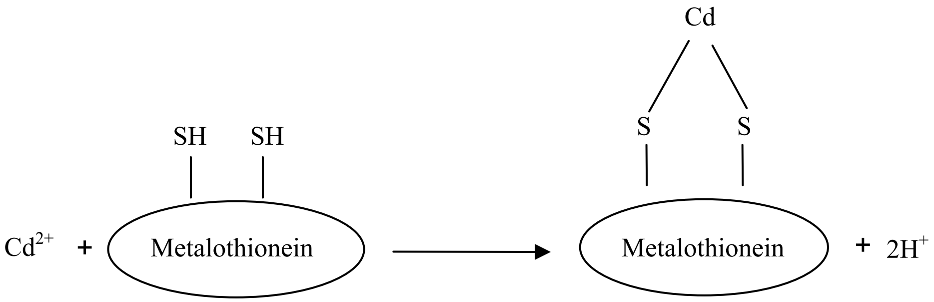

Figure 2. Cadmium-metallothionein interaction.

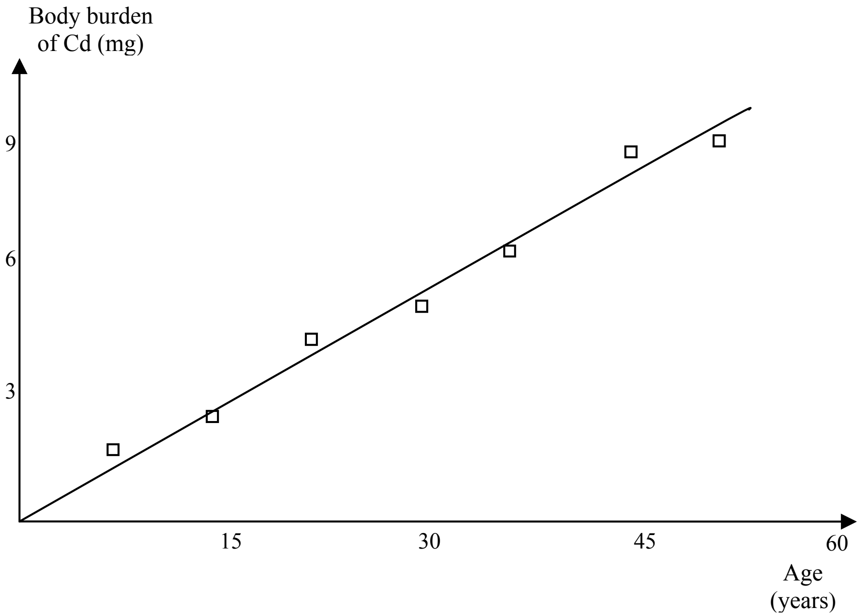

Figure 3. Cadmium accumulation with age in humans.

Newborns have the highest Cd absorption rate through milk. Cadmium uses the same transport system in organism as calcium (calcium-binding protein). Because children's bodies need calcium for their growth and development, high amounts of calcium-binding protein are biosynthesized.

In cadmium intoxication, cadmium substitutes for calcium, and the effect is attributed to displacement of calcium from its action sites in the neuromuscular junctions.

Cadmium toxicity is mediated by the formation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl and peroxyl radicals. ROS are highly toxic and can oxidize lipids, proteins and nucleic acids, thus causing inactivation of enzymes, membrane damage and lipid peroxidation (Franco et al., 2008).

3. Lead Exposure and Its Effects

Exposure to inorganic lead or its inorganic/organic compounds occurs in environmental or occupational circumstances (Vinodhini and Narayanan, 2008).

The primary source of environmental lead in many countries was the combustion of leaded gasoline. Being an important pollutant, lead was listed as one of the six major air polluting metals by US Environmental Protection Agency (EPA).

Lead has a high affinity for the sulfhydril groups of enzymes, to form mercaptides (adenylcyclases, aminotransferases).

In the human organism, lead may cause neurological damage, kidney damage, hematopoietic system damage and abdominal pain. In heme biosynthesis, lead affects four enzymes: δ-aminolevulinic acid synthetase, δ-aminolevulinic acid dehydrogenase, coproporphyrinogen oxidase, ferrochelatase. In heme catabolism lead affects heme oxidase which catalyzes the formation of bilirubin (Williams et al., 2000). The result of lead poisoning is the accumulation of δ-aminolevulinic acid (ALA) in serum and urine, as well as protoporphyrin IX in red blood cells (Timbrell, 2002).

Lead interferes at several steps in the iron uptake into the mitochondria, with the utilization of iron in erythrocytes and with globins synthesis. It also inhibits or mimics the action of calcium.

The mechanism of human lead poisoning is based, as in the case of cadmium, on the inhibition of thiol-dependent enzymes.

Lead-based paint (dye) present in old houses is very dangerous because children often ingest it from walls, woodwork, plaster, floors and furniture. For young American children living in urban areas, subclinical lead poisoning is the most common and serious environmental disease (Ming-Ho, 2005). More than

2.2% of US children with ages between 1 and 5 years still have blood lead concentrations above 10 µg/dL and more than 17% are at risk of lead poisoning.

In adults, about 5–10% of ingested inorganic lead is absorbed, whereas in children the values may be higher than 50% (Figure 4).

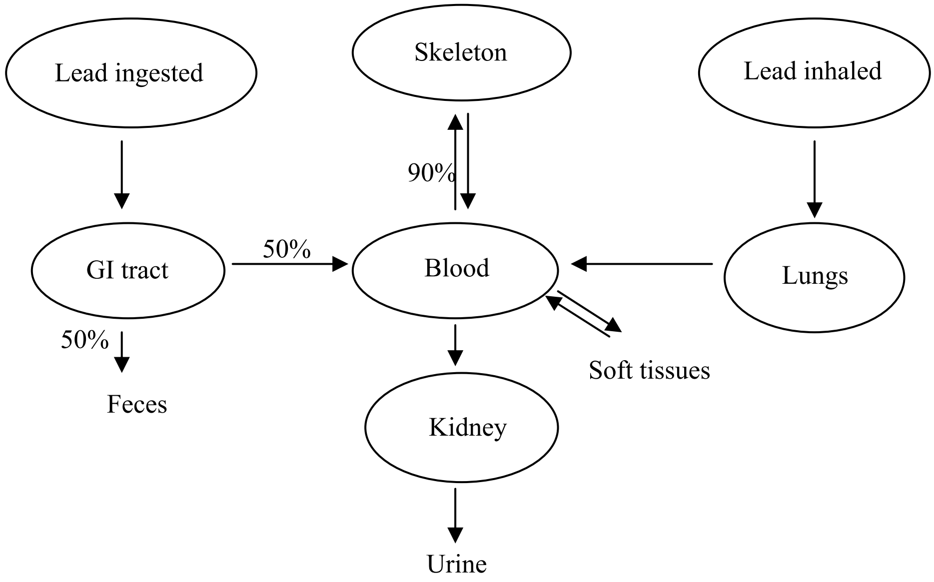

Figure 4. Lead metabolic circuit in children.

Most of the lead in the bloodstream (90%) is deposited and accumulated in children's skeleton. Newborns and children have been shown to be more susceptible to lead poisoning than adults. Moreover, lead can pass through the placental barrier and may reach the fetus, causing abortions and stillbirths.

Effects of lead on children's bodies are different from its effects in adults (there are partial similarities), because children's metabolism (absorption, transport, distribution, reactions) is more rapid (Mirsa and Pandey, 2005; Hoornweg, 2000). Comparative lead effects on human health are presented in Table 1 (Davidova, 2005).

To be noticed that some of the effects, like hemoglobin decrease, anemia, and encephalopathy are induced in children at lower concentrations than in the adults. Moreover, if lead concentrations lower than 10 µg/dL have no effects in adults, they do affect children's physical state. At concentrations between 90 and 120 µg/dL lead induces encephalopathy in adults, but death in children.

TABLE 1. Comparative lead effects on human health.

Lead concentration (μg/dL)	Effects on children	Effects on adults
>10	IQ decrease Physical state alteration	No effect
10–30	Vitamin D decrease	Blood pressure increase
30–50	Hemoglobin decrease	Nephropathy
50–70	Anemia	Hemoglobin decrease
70–90	Encephalopathy Nephropathy	Anemia
90–120	Death	Encephalopathy

4. Arsenic Exposure and Its Effects

The main source of arsenic intoxication for humans is from polluted air. The release of arsenic into the atmosphere is dominated by anthropogenic emissions, from combustion of coal and incineration of wood products, as sulfides oxidated to arsenic oxide (Sarkar, 2008). The excretion of arsenic is mainly via urine.

Pentavalent arsenic does not react with the active sites of enzymes directly, being not toxic. It should be firstly reduced to trivalent form before exerting its toxic effects. Arsenic combines with sulfhydryl groups on animal proteins (nail, skin and hair) with high selectivity. Biotransformation of inorganic arsenic in some animal species, using S-adenosylmethionine as a methyl donor group, leads to highly toxic trivalent methylated arsenicals (adaptation mechanism).

Arsenic has predilection for skin and hair. From skin, arsenic is excreted by desquamation and sweat. Arsenic present in hair is used as a biomarker for toxic exposure.

Arsenic acts to coagulate proteins, forms complexes with coenzymes and inhibits the biosynthesis of cellular ATP (Manaham, 2003). ATP is an universal biocompound that accumulates and releases metabolic energy. Glyceraldehyde-3-phosphate is an glycolytic intermediate compound, that combines with the oxidized form of nicotinamide adenine dinucleotide (NAD^+) and inorganic phosphate, to form an acyl phosphate (1,3-bisphosphoglycerate). 1,3-Bisphosphoglycerate is a compound with high potential for donating the phosphate groups (macroergic compound), whose free energy of hydrolysis can be coupled to the ATP synthesis. Due to their structural similarities, arsenic substitutes phosphorus in 1,3-bisphosphoglycerate and leads to the synthesis of a compound with low potential for transferring the phosphate groups (adverse metabolic effects). This stops ATP formation from ADP. Inactivation of pyruvate dehydrogenase will prevent generation of ATP and the inhibition of succinic dehydrogenase can uncouple oxidative phosphorylation, a process that results in the disruption of all cellular functions.

Acute arsenic toxicity is characterized by vomiting, bloody diarrhea, pulmonary edema, hemorrhagic bronchitis, hypotension, tachycardia, anemia, leucopenia, and death in patients that have ingested large doses of arsenic.

Several epidemiological studies report acute health effects from subchronic arsenic exposure (from drinking water) in children: gastrointestinal, neurological and skin effects, facial edema, dermatoses and cardiac arrhythmia (Tsuji et al., 2004).

5. Synergistic Effects of Cadmium, Lead and Arsenic

Human exposure to environmental pollutants is characterized as co-exposure, that leads to effects that may be mediated by genetic factors, doses and exposure duration. This produces not only additive effects, but may also produce new, synergic effects that are not seen in single element exposure (Wang and Bruce, 2008).

Studies of human exposure to arsenic, cadmium and lead mixtures, have demonstrated interactive effects on human organs and more severe renal effects than exposure to each metal alone (synergistic effects). The most affected organ is the kidney (swelling of proximal tubule, alteration of morphology, increase of urinary excretion of porphyrins) which are reflected in:

- proteinuria (Hong et al., 2004; Nordberg et al., 2005);
- high concentration α-glutathione transferase in urine (Garcon et al., 2004; Garcon et al., 2007);
- low concentration glutathione peroxidase in urine (Wasowicz et al., 2001);

A significant nonlinear relationship was identified between the mental retardation and developmental delays of children, and levels of arsenic and lead in soil samples, taken near their mother's residence, during pregnancy (Liu et al., 2010).

Accumulation of arsenic, cadmium and lead in ecosystems is a continuous source of exposure for humans. The presence of these metals in the human body leads to the development of some common action mechanisms: reaction with sulfhydryl protein groups (enzyme inactivation), interference with essential elements (Zn, Cu, Se, Ca, Fe) and production of reactive oxygen species (ROS). ROS (hydrogen peroxide, superoxide anion, hydroxyl radical, peroxyl radical) oxidize some biomolecules (proteins, lipids, nucleic acids), causing oxidative stress: enzyme inactivation; membrane damage; lipid peroxidation (Figure 5); alterations in regulatory cell mechanisms; DNA damage; death.

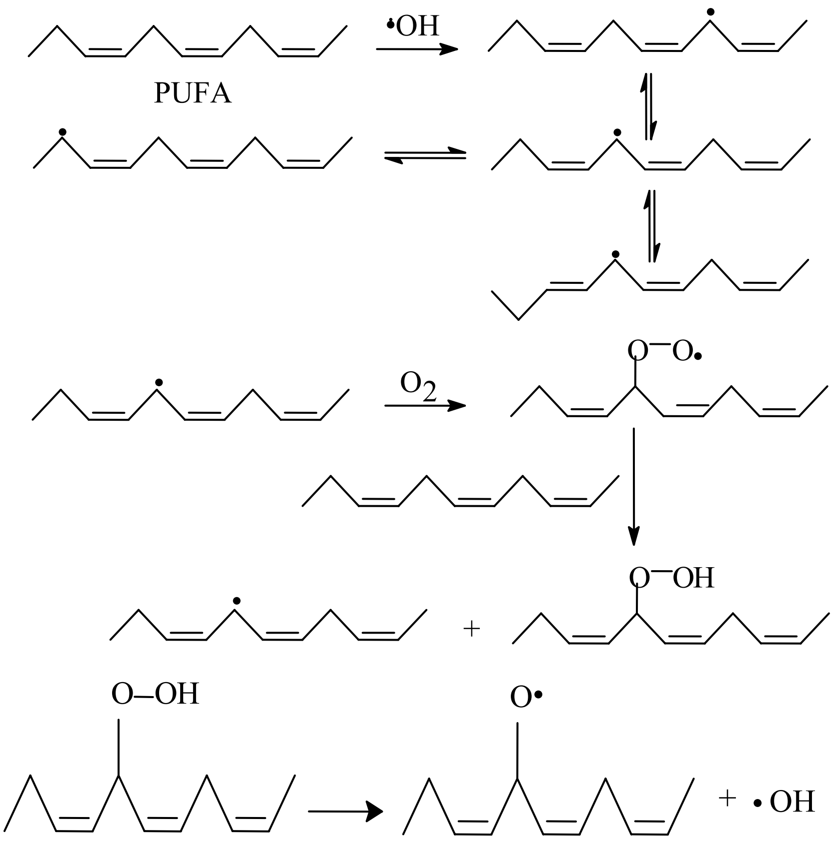

Figure 5. Interactions of poly-unsaturated fatty acids (PUFA) with ROS and hydroperoxid compounds formation.

Oxidative stress can be an effect of heavy metals mixtures, because mitochondria (major intracellular source of ROS), are common targets for arsenic, cadmium and lead. These heavy metals affect antioxidant enzymes (superoxide dismutase, catalase, peroxidase) in which the active sites contain essential metals (Cu, Se, Zn) or sulfhydryl groups.

Studies showed that ROS generation, associated with arsenic, cadmium and lead exposure, play a fundamental role in the induction of some health effects: diabetes, arteriosclerosis, hypertension, cancer (lung, liver, kidney, bladder, skin) and neurological diseases (Xu et al., 2008; Ruiz et al., 2009).

The imbalance between the levels of the essential metals, on one part, and arsenic, cadmium and lead, on the other part, contributes to excessive ROS production, causing a disturbance in the pro-oxidant/antioxidant ratio, and affecting children's development and fetal growth (Llianos and Ronco, 2009).

6. Conclusions

This review describes the penetration, transport, metabolism and excretion, of arsenic, cadmium and lead, in the human body in general, and in children's bodies in particular, as well as their effects. The individual effects of these three heavy metals and their synergistic effects were discussed.

From the synergistic action (cocktail) is difficult to discriminate individual actions and effects. The effects of exposure and co-exposure in sensitive populations (elderly, pregnant women and children) appear at much lower levels than in the rest of the adult population.

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EVOLVING UNDERSTANDING OF THE RELATIONSHIP BETWEEN MERCURY EXPOSURE AND AUTISM

THOMAS A. LEWANDOWSKI[1, 2]*
[1] *Department Health and Nutrition Sciences*
Brooklyn College CUNY, 4135 Ingersoll Hall
2900 Bedford Avenue, Brooklyn, NY 11210, USA
[2] *Gradient LLC*
600 Stewart Street, Seattle, WA, 98101 USA

Abstract. Over the last decade autism and autistic spectrum disorders (ASD) have become an increasing focus of scientific research and public interest. The idea that mercury exposure may play a causative role in autism was first suggested in connection with postnatal exposures to ethylmercury via vaccination. This hypothesis has not been supported by the results of several large epidemiology studies and scientific reviews but nonetheless the notion that mercury in vaccines causes autism has remained fixed in the public mind. Several ecological studies have also suggested that inorganic mercury exposures from environmental (i.e., non-vaccine) sources are correlated with autism prevalence. To better understand some of the limitations of these ecological analyses, and to explore the robustness of the mercury–autism hypothesis, we used data collected for the U.S. State of Texas to examine whether other surrogate exposure measures (e.g., emissions of other chemicals, downwind status from a power plant, local fish consumption advisories) and different analytical approaches led to similar findings. Using multilevel Poisson regression analysis we found that air mercury emissions reports were significantly associated with same year autism prevalence in Texas school districts using data obtained for 2001 and 2002 but that associations were not significant using data from 2003 to 2005. Evaluations using other surrogate exposure variables (i.e., downwind location from coal-fired power plants or existence of mercury related fish consumption advisories) also did not yield statistically significant associations. We did observe that the treatment of censored data had an important effect on the analysis; results which were statistically significant when censored data were handed as zeros became non-statistically significant when they were treated as the mid-point of the censored range. This indicates that what is required to clearly answer questions about

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* To whom correspondence should be addressed: TLewandowski@brooklyn.cuny.edu

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environmental mercury and autism is a well controlled case-control or cohort study with extensive pre- and post-natal mercury exposure assessment. Government agencies, advocacy groups and mercury emitting industries should all consider supporting such studies so that this contentious health concern can be definitively addressed.

Keywords: Mercury, autism, environmental epidemiology

1. Autism

Autistic disorder, commonly called autism, is a health condition that has changed from being a relatively unknown disease to a topic of increasing focus for researchers and the public, particularly over the past decade (Ozonoff, 2001; Singh et al., 2009; NIEHS, 2010). Autistic disorder is one of three related Autistic Spectrum Disorders (ASD), the others being Asperger's Syndrome and Pervasive Developmental Delay-Not Otherwise Specified (PDD-NOS).[1] All ASDs are characterized by three key traits: (1) behavioral deficits in the areas of social interaction, (2) deficits in communication and (3) repetitive and/or restrictive behaviors such as an inability to tolerate even minor changes in the home environment (APA, 2000; Schreibman, 2005; Scahill and Bearss, 2009). The specific presentation and severity of the symptoms in each of these three key areas may vary somewhat among the three conditions. For example, unlike children with autism, children with Asperger's Syndrome typically have minimal delay in development of general language abilities although their use of language may be atypical (APA, 2000). The deficits in social interaction are fairly unique to the ASD among developmental diseases. These may manifest as a lack of empathy or intuition about thoughts and feelings of others, skills that are necessary for an individual to function in human society (Campbell, 2004; Schreibman, 2005). In the frequently quoted words of one notable person with autism, University of Colorado Professor Temple Grandin, being a person with autism is like being "an anthropologist on Mars" (Sacks, 1993), i.e., trying to function in a society without a clear concept of what constitutes normative behavior.

Unlike some other neurological conditions (e.g., Down's syndrome, Tay - Sachs disease), ASDs cannot be diagnosed by the presence of any biological marker and must be diagnosed based on behavioral observation rather than

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[1] Although not officially sanctioned, the term ASD is in widespread use. The three disorders encompassed by the term ASD are part of the formal diagnostic category of Pervasive Developmental Delay (PDD), along with the conditions Childhood Disintegrative Disorder and Rett's Syndrome.

laboratory testing (Landrigan, 2010). It requires a phenomenological diagnosis (Schreibman, 2005). A number of standard diagnostic instruments (interviews or observational guidelines) are available for the diagnosis of autism, each of which has its own particular strengths and weaknesses, as reviewed by Mayes et al. (2009) and Oosterling et al. (2009). Consistent with the variable nature of symptoms seen across the population of individuals with autism, the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) requires for diagnosis that individuals being evaluated display at least six of a broader set of 12 described behavioral traits (i.e., at least two related to social interaction, one related to communication, and one related to restricted or repetitive behavior) as well as an indication of developmental delay in social interaction, communication, or abstract thinking (APA, 2000).

The first symptoms of autism are usually apparent by the end of the first 3 years of life (CDC, 2009), although some children appear to develop normally beyond this window and then later regress. Early symptoms may include lack of eye contact, failure to interact with parents in an emotional way (e.g., failing to treat the parent as a source of comfort and security), delays in language development, and prolonged echolalia (Schreibman, 2005). Symptoms in older children may include a failure to play with toys in typical ways, peculiar and often repetitive movements (e.g., hand flapping), compulsive behaviors (e.g., repeatedly lining up objects in single file) and a resistance to changes in environment (e.g., requiring that objects in the living environment not be moved) (Schreibman, 2005). Although various strategies have been developed to help individuals with autism function more easily in society, there is currently no cure for autism.[2] For those individuals with the most severe symptoms, the impact on their lives and those of their families can be substantial and devastating.

Autism as a diagnostic term ("Autistic disturbances of affective contact") was first used by psychiatrist Leo Kanner in 1943 to describe children who seemed withdrawn and otherwise disengaged from the world around them (Kanner, 1943). Since that initial description, the diagnostic criteria for autism have changed as have theories about its etiology. Psychosocial causes predominated in the 1950s and 1960s and included the "refrigerator mother" theory espoused by psychoanalysts such as Bruno Bettleheim. This was based in part on Kanner's early observation that parents of autistic children seemed indifferent to their children (Kanner, 1949; Bettleheim, 1967). The 1970s saw the publication of several studies showing the genetic and heritable nature of the condition (Rutter and Bartak, 1971; Ornitz, 1973; Folstein and Rutter, 1977

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[2] Various cures are currently claimed but have not been verified by scientific studies. Unfortunately, the field of autism research has a long history of claims of cures which have been later found, once subjected to rigorous testing, to be ineffectual.

a, b) and a rejection of psychosocial causation theories. More recently, research has focused on identifying potential environmental (broadly defined) causes, as discussed below. Much of the recent research on autism has been encouraged or championed by parental organizations, which have also done much to decrease the stigma associated with the disease and to ensure that support services are available through local school systems.

1.1. CHANGING PREVALENCE OF AUTISM

Autism was formerly considered to be a fairly rare disease to the extent that it was not well known among the general public (Ozonoff, 2001). A number of studies published in the 1970s yielded prevalence estimates in the range of 5 per 10,000 individuals (Wing, 1993; CDC, 2008). By 1990, the prevalence of autistic disorder was reported to be roughly 2 cases per 1,000 births, while the prevalence of ASD was estimated at 5.5 cases per 1,000 births (Atladóttir et al., 2007). A more recent U.S. CDC report indicated a prevalence rate for ASD in the U.S. of 1 in 110 children (CDC, 2009). Note that diagnostic criteria for autism changed over this time frame and that the broader term ASD only came into use in the late 1980s (CDDS, 2003). Thus it is important to be clear when comparing prevalence estimates whether they refer to autistic disorder specifically or the more general ASD. Due to changes in diagnostic criteria, such comparisons are problematic and the broadening in criteria likely contributes to some extent to the apparent increase in autism prevalence (Hertz-Picciotto and Delwiche, 2009). In addition, there is evidence for a role for changing diagnostic preference (Shattuck, 2006). For example, using data from California, Croen et al. (2002) demonstrated that from 1987 to 1994 an increase in the prevalence of "full syndrome autism" occurred in parallel with a decrease in the prevalence of mental retardation, suggesting diagnostic substitution. A number of researchers have also suggested that a decrease in the stigma associated with autism, coupled with an increase in available special education services for autistic children and better screening programs for early detection, have lead to a greater willingness for psychiatrists and psychologists to diagnosis developmental problems as autism (Nassar et al., 2009). However, in a recent analysis of autism prevalence data from California, the authors claim that the observed increase in autism prevalence cannot be completely explained by factors such as earlier case ascertainment, changes in diagnostic criteria, and inclusion of milder cases (Hertz-Picciotto and Delwiche, 2009). Thus the question of whether the observed increase in autism prevalence represents a true biological phenomenon remains a controversial topic, one that is unlikely to be settled in the near future. What is clear is that the sense of anxiety or anger created by the possibility of an "epidemic of autism" contributes to the acrimony and challenge of conducting research in this area.

1.2. KNOWN AND SUGGESTED CAUSES OF AUTISM

Children with autism display physiological differences from typically developing children (for example larger brain size and brain cell numbers) which clearly suggest a biological basis for the disease (Santangelo and Tsatsanis, 2005; Acosta and Pearl, 2003). These differences suggest that like most other neuro-development diseases, autism is due to events which occur in the prenatal time period, during the period of brain organogenesis, but with behavioral effects which are only apparent post-natally. This in turn suggests that post-natal exposures (e.g., from vaccinations) are unlikely to be a cause of autism although this does not rule out the possibility that some postnatal event could provide an accessory trigger in those with prenatally-derived predispositions.

As discussed above, the substantial role of genetics in the etiology of autism has been known since the late 1970s. For example, studies of identical twins have suggested a concordance rate for autism between 60 and 90% (Folstein and Rutter, 1977a, b; Schreibman, 2005). Even non-twin siblings of individuals with autism have a 50 to 100-fold increased likelihood of being diagnosed with autism compared to the general population (Schreibman, 2005). It has also long been recognized that autism occurs 4–5 times more often in boys than girls, suggesting the involvement of some gene associated with the sex chromosomes (CDC, 2010). More recently, studies have shown that parents of children with autism themselves display some mildly autistic traits (e.g., difficulties in communication), suggesting a complex genotype that varies in the extent of its expression (Ruser et al., 2007; Losh and Piven, 2007; Adolphs et al., 2008).

Based on several decades of research, it is now clear that autism is not caused by a single gene but by a number of genes acting together in combination. This multi-gene concept is consistent with the highly variable symptom pattern. It is also increasingly accepted that autism may be caused by the interaction of genetic and non-genetic factors. This would help to explain the lack of complete concordance in twin studies and also the possible increase in prevalence (because the prevalence of a disease with a purely genetic etiology would be unlikely to change over the span of mere decades but would require hundreds if not thousands of years). Various non-genetic factors which have been associated with autism include specific infectious diseases, environmental pollutants, parental age, smoking, certain illicit and prescription drugs, and complications during pregnancy (Davis et al., 1992; Gardener et al., 2009; Landrigan, 2010). Among these non-genetic factors, the possible role of environmental pollutants (and in particular metals like mercury) has elicited particular interest.

2. Mercury in The Environment

Mercury is a ubiquitous part of the environment with both natural and anthropogenic sources. Natural sources include volcanism and degassing of the earth's crust while anthropogenic sources of mercury include industrial discharges, waste incineration and burning of fossil fuels, chiefly coal (ATSDR, 1999). Anthropogenic sources likely contribute more than sixty percent of the total mercury released annually to the environment (ATSDR, 1999). Mercury emissions may travel far in the environment, up to thousands of miles from their point of origin, depending on chemical species and emissions conditions (Seigneur et al., 2004). Thus among all metals, mercury is truly a pollutant of global scale which requires global cooperation. Of particular concern are the increasing mercury emissions from developing nations in Asia, which in 2005 contributed 66% of the world's anthropogenic mercury emissions (UNEP, 2008). In the absence of more stringent control technology, Asian air mercury emissions are projected to increase 30% by 2020 (UNEP, 2008).

Mercury exists in three principal forms: elemental mercury, inorganic mercury salts and organic mercury. Organic mercury consists of both alkylated (e.g., methyl, dimethyl, ethyl) and aromatic (e.g., phenyl) species. Conversion between the inorganic and organic forms occurs in the environment, primarily in aquatic sediments (Hamdy and Noyes, 1975; Hines et al., 2000). The chemical form of mercury is critical to predict the movement of mercury in the environment as well as its effects on biological organisms (Gochfeld, 2003; Clarkson and Magos, 2006).

2.1. EVIDENCE OF NEURODEVELOPMENTAL EFFECTS

A dramatic manifestation of the toxic effects of methylmercury (MeHg) exposure occurred from 1953 to 1971 in the areas surrounding Minamata Bay in Japan (Takeuchi et al., 1959; Takeuchi, 1982; Tsubaki and Irukayama, 1977; Harada, 1995). The exposures resulted from ingestion of MeHg contaminated fish. The source of the pollutant was eventually traced to an acetaldehyde plant that discharged effluent directly to the bay (Harada, 1995). While nervous system effects were observed in exposed adults, the most striking effects were seen in newborn infants. CNS and other developmental effects were observed in infants with relatively unaffected mothers, pointing out the special sensitivity of the human fetus to MeHg (Harada, 1995; Choi, 1989). The effects in newborns were described as a nonspecific cerebral palsy coupled with motor, sensory and cognitive deficits (Tsubaki and Irukayama, 1977). A second outbreak, with similar exposures and symptoms, occurred in the nearby area of Nigata in 1964 after plant effluent was redirected to a nearby river (Harada, 1995).

Another large outbreak of MeHg poisoning occurred in Iraq in 1971–1972 (Bakir et al., 1973; Amin-Zaki et al., 1976). Over 6,500 individuals were hospitalized and 459 died after having been exposed (Bakir et al., 1973) when seed grain coated with a fungicide containing both methyl- and phenylmercury was used to make bread due to inappropriate labeling. The neurodevelopment toxicity seen in the Iraqi victims was very similar to that seen in Japan and reinforced the serious consequences of high level exposure to MeHg for the developing fetus. Less extensive outbreaks of MeHg poisoning have been reported in the U.S. (Curley et al., 1971), the former Soviet Union, Pakistan, Sweden and Guatemala (Elhassani, 1982). No studies reporting increased autism prevalence in any of the populations experiencing high level MeHg exposures were located in the scientific literature.

Several studies have suggested that even low levels of MeHg in fish coupled with high fish consumption diets may lead to subtle neurological deficits in the developing human (Grandjean et al., 1997; McKeown-Eyssen and Ruedy, 1983; Burbacher et al., 1990), although contrary findings have also been reported (Myers et al., 1995a, b; Myers et al., 1997; Davidson et al., 1998; Davidson et al., 2006). These effects (i.e., an association between methylmercury exposure and deficits in neurological test performance) are currently used as the basis for mercury regulation in the U.S. and many other countries. It should be noted that the neuropsychological effects observed in these studies are seen on the population level but cannot be observed on an individual basis. The two key study populations used to assess low level MeHg exposures (i.e., the Faroe Islands and the Seychelle Islands) have also been examined for elevated levels of autism. For example, Ellefsen et al. (2007) reported that autism prevalence was not different from that of other Western countries among children in the Faroe Islands (Ellefsen et al., 2007), despite the fact that exposure levels (i.e., to MeHg via fish consumption) are higher than those encountered in most of the United States (Oken et al., 2005). Higher frequencies of autism have also not been noted in the Seychelle Islands cohort, which has been subjected to a battery of cognitive, behavioral and attention tests (Myers et al., 2003).

With respect to elemental mercury, the neurological effects are also well recognized. Unlike MeHg, elemental mercury is poorly absorbed across the GI tract. The exposure route of concern is inhalation because elemental mercury is absorbed quite readily across the alveolar membrane (Gochfeld, 2003). The key symptoms associated with elemental mercury exposure are different from those observed with organic mercury. These primarily involve tremor and other peripheral neuromuscular effects, changes in mood and mental state (e.g., excitability, irritability, depression), and loss of cognitive ability (Hamada and Osame, 1996; U.S. EPA, 2009). Environmental exposures to elemental mercury above health criteria are uncommon; the ATSDR in the U.S. estimated that average intake of elemental mercury via inhalation was 0.030 µg/day (ATSDR, 1999). For comparison, the U.S. EPA's reference dose for elemental mercury (0.009 mg/m^3) suggests a safe level of exposure for a typical child could be as high as 90 µg/day.

The primary target organ for the inorganic mercury (mercurous, mercuric) salts is the kidney although effects on other organ systems including the CNS may occur (Hamada and Osame, 1996). Due to the use of inorganic mercury compounds as antimicrobial agents used for young children, for example in teething powders in the early twentieth century, a particular inorganic mercury syndrome called acrodynia in children has been described (Hamada and Osame, 1996).[3] The common symptoms of acrodynia include redness of the skin of the feet, hands and face, neuropathy in the extremities, inflammation of the gum tissue and potential loss of teeth, listlessness, drowsiness, irritability, anorexia, photophobia, and difficulty walking (Hamada and Osame, 1996; ATSDR, 1999). The involvement of the immune system in the etiology of acrodynia has been posited (Hamada and Osame, 1996). Note that the characteristic symptoms of acrodynia are the peripheral nerve effects and skin redness (ATSDR, 1999), two features which are not characteristic of autism.

In summary, chemical species is important not only for understanding the fate and transport of mercury in the environment but also for predicting the toxicological effects that will arise with sufficient exposure. Although all mercury compounds are capable of causing neurotoxicity, it is the organic forms (primarily MeHg) that are known to particularly target the developing nervous system. The neurological effects of this form of mercury which are reported in recent large epidemiology studies are observable only on a population level and, unlike autism, are not observable on the individual level. Thus the existing toxicological literature concerning the health effects of mercury compounds provides little support for the hypothesis that mercury exposure can cause autism.

3. Autism, Vaccines and Mercury

The idea that mercury exposures could be associated with autism was first suggested in the context of postnatal exposures to ethylmercury from vaccines (Bernard et al., 2001). Ethylmercury (thimerosal) had long been used in some multi-dose vaccine preparations as a preservative (Aschner and Ceccatelli, 2009). Throughout the 1980s and 1990s, the recommended childhood vaccination schedule increased, leading to increased episodic ethylmercury exposures (IOM,

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[3] Acrodynia may also occur in adults and may be caused by elemental mercury exposure. However it has most frequently been observed as a result of in inorganic mercury salt exposures in children.

2004). One difficulty in assessing the risks of this exposure was the lack of a clear exposure criterion for ethylmercury. Lacking such a value, the cumulative ethylmercury dose from 3 to 5 shots was compared to the chronic reference dose for MeHg, which is intended to be protective for repeated daily exposure. Not surprisingly, the comparison showed that the cumulative dose of ethylmercury was above the reference dose for methylmercury (Ball et al., 2001).[4] The fact that the two forms of mercury are different and that the exposure patterns are quite distinct makes such a comparison questionable (Aschner and Ceccatelli, 2009). Nonetheless, the results of the comparison caused alarm among some regulators and members of the public.

A number of small scale analyses (e.g., of data from vaccine outcome databases) subsequently suggested an association between postnatal thimerosal exposure and autism (Geier and Geier, 2003; 2006a, b; 2007; Young et al., 2008). However, the hypothesis has not been supported by the results of large-scale studies conducted in Denmark (Madsen et al., 2003), the United Kingdom (Andrews et al., 2004) or the United States (Thompson et al., 2007; Schechter and Grether, 2008). The available epidemiological literature has also been reviewed in a number of articles (Parker et al., 2004; Aschner and Ceccatelli, 2009) as well as by authoritative bodies (IOM, 2004; AAP, 2008), all of whom have rejected the idea that thimerosal in vaccines causes autism. The lack of an association is also confirmed by the continued increase in autism cases even after the removal of thimerosal from vaccines (Figure 1). Yet the subject remains of great media interest and the belief that "vaccines cause autism" is prevalent among the general public (DeStefano, 2007; Baker, 2008).

Parents groups as well as governmental agencies have funded a number of research projects aimed at trying to understand how chemicals such as mercury could produce autism. A number of these studies suggest that autistic children exhibit altered metabolism and increased susceptibility to chemicals capable of producing oxidative stress (James et al., 2004; James et al., 2006; Zoroglu et al., 2004; Williams et al., 2007).

Mercury certainly has the induction of oxidative stress as one of its purported mechanisms of action, yet many other chemicals, including many of the endogenous products of metabolism, also have this potential. A recent detailed analysis of blood mercury concentrations in 249 children with autism and 149 typically developing children found no difference between the two groups (nor with nationally representative samples), although the authors note that their study did not assess pre-natal exposures (Hertz-Picciotto et al., 2010). The role for

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[4] The reference dose (RfD) is the U.S. Environmental Protection Agency's estimate of a chronic daily intake that is unlikely to cause adverse effects, even in sensitive populations.

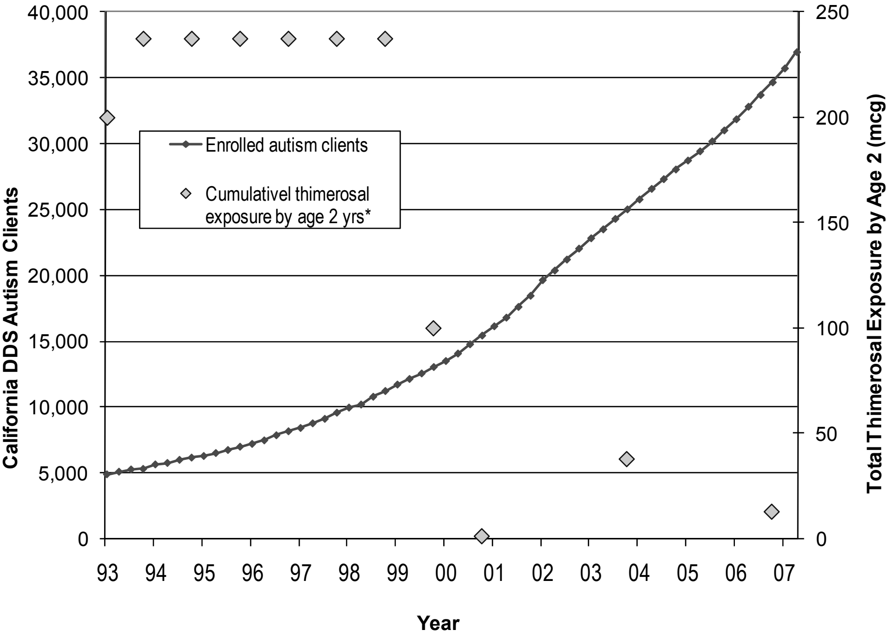

Figure 1. Increase in Autism Clients Served by the California Department of Developmental Services versus Thimerosal Content of U.S. Vaccines. Autism data were obtained from quarterly case load reports published by the California Department of Developmental Services (CDDS). Maximum cumulative thimerosal exposure from the recommended vaccination schedule in the U.S. up to age 2 was estimated using vaccine content data collected by the Massachusetts Department of Environmental Protection (MDPH, 2007) and vaccine schedule information from the U.S. Centers for Disease Control (CDC, 2006).

early-life immune system insults (including those produced by metals) as a causal factor in autism has also been explored in detail by Dietert and Dietert (2008). Recently, a genomics study of autistic and typically developing children has shown that a number of genes associated with cell morphology and cell death are differentially expressed in autistic children and that expression of these genes is correlated with blood mercury levels (Stamova et al., 2009). Some authors have speculated that autistic children (or a subset of autistic children) have genetic susceptibility to chemicals like mercury and that, in such children, mercury exposures may serve as a trigger for otherwise latent autism (James et al., 2006; Williams et al., 2007; Landrigan, 2010). However, the extent to which the cellular and molecular effects observed in these studies have a role in the causation of autism versus being downstream consequences of the disease itself is not clear. The cited studies do suggest autistic children may be at higher risk for adverse effects subsequent to chemical exposure.

4. Autism and Environmental Mercury

4.1. EARLIER STUDIES

Beyond the subject of vaccines, several ecological studies have suggested that inorganic mercury exposures from environmental sources may be associated with autism prevalence (Palmer et al., 2006; Palmer et al., 2009; Windham et al., 2006). Windham and colleagues conducted a study looking at environmental exposures and autism prevalence in Northern California (Windham et al., 2006). The study examined 284 children with ASD and 657 controls, born in 1994 in the San Francisco Bay region. Children with ASD were identified from records of the state health care agency as well as those of a major health insurer. Potential exposures to 19 chemicals of interest were estimated using data obtained from the U.S. EPA Hazardous Air Pollutants (HAP) database and maternal address information recorded on birth certificates. The authors found that adjusted odds ratios for ASD were elevated in population quartiles with the highest predicted exposures to chlorinated solvents and heavy metals. The individual chemicals contributing to the estimated increased risk included mercury, cadmium, nickel, trichloroethylene, and vinyl chloride. The adjusted odds ratio of mercury and autism in the highest quartile group was 1.92 (95% confidence interval: 1.36–2.71). A limitation of this study is that the estimated air mercury concentrations between the ASD cases (0.0008 ± 0.0019 $\mu g/m^3$) and controls (0.0006 ± 0.001 $\mu g/m^3$) were both quite low and had considerable overlap. This makes it difficult to assess the existence of a dose-response relationship. In addition, the potential impact of uncontrolled confounders is difficult to ascertain, as the authors themselves acknowledge, and the reliance on HAP data to estimate individual exposure (including exposures inside the home), also limit the ability to draw definite conclusions from the results.

A publication by Palmer et al. in 2006 reported finding that mercury emissions in for the U.S. State of Texas were associated with local autism prevalence. The authors obtained mercury emissions data for 2001 from the U.S. EPA's Toxic Release Inventory (TRI) database, which collects chemical emissions data from industrial facilities across the U.S. Note that mercury emissions listed in the TRI database are almost entirely limited to inorganic mercury (EPA, 2010). Autism count data were obtained for local school districts from the Texas Education Agency (TEA). Along with various demographic variables, the autism and mercury emissions data were included in a Poisson regression model to see if mercury emissions were a predictor of autism prevalence. The authors reported that such an association was found for the 1 year studied, noting that "for each 1,000 lb of environmentally released mercury, there was …a 61% increase in the rate of autism." A number of issues with this analysis were subsequently noted (Lewandowski, 2006). For example, TRI data likely do not reflect actual concentrations present in an individual's environment. This is particularly likely for chemicals like mercury which undergo fairly complicated fate and transport processes. Air modeling studies indicate that mercury deposition that occurs in the west of the nation (including Texas) is overwhelmingly attributable to Asian or other non-U.S. sources (Seigneur et al., 2004). Moreover, most of an individual's mercury exposure comes from fish consumption (as MeHg), primarily of ocean caught fish (Carrington and Bolger, 2002; Dabeka et al., 2004) and mercury levels in such fish would not be correlated with local emissions. Thus it is very unlikely that a population's overall mercury exposure would be substantially altered by a nearby air source to the extent that it would produce statistically detectable effects.

4.2. REANALYSIS OF THE TEXAS DATA (LEWANDOWSKI ET AL., 2009)

To better understand some of the limitations of these earlier analyses, and to explore the robustness of the mercury–autism hypothesis, we examined whether other surrogate exposure measures (e.g., emissions of other chemicals, downwind status from a power plant, local fish consumption advisories) and other analysis methods produced similar findings (Lewandowski et al., 2009). Using multilevel Poisson regression analysis and data obtained from a number of publicly available databases, we explored the possible association of a number of different variables with school district autism prevalence in Texas. The analyses included:

- Evaluating the relationship of school district autism prevalence with same year TRI air mercury emissions. This was conducted for multiple years, from 2001 through 2005.
- Air mercury data were also used in a latency analysis wherein a 4–5 year time period was allowed between potential in utero exposure and autism case ascertainment.
- Evaluating associations with air emissions data for various other chemicals (solvents, other metals).
- Evaluating associations of school district autism prevalence with various surrogate measures for mercury exposure (i.e., downwind location from a coal fired power plant and the existence of mercury-related fish consumption advisories in the local area).

Autism counts and other student demographic data were obtained from the TEA. Student data which were censored due to small numbers and privacy policies were assigned a value of zero or the midpoint of the censored data range, as described in Lewandowski et al. (2009). TRI data were obtained from the U.S. EPA TRI website (U.S. EPA, 2010) and other relevant data (wind data, fish consumption advisories) were obtained from on-line data bases (Lewandowski et al., 2009).

Our analysis found that air mercury emissions data were significantly associated with autism prevalence in Texas school districts for 2 years (2001 and 2002) when censored student data were treated as zeros, but not when they were treated as the midpoint of the censored data range (Table 1). Regardless of the data treatment approach, associations were not statistically significant using data from 2003 to 2005. A significant association was also not observed using the more biologically plausible approach of evaluating mercury emissions data for 2000 or 2001 and autism prevalence data for 2005–2006 or 2006–2007, thus accounting for the latency effect of case ascertainment. These results are perhaps not surprising because mercury emissions in Texas appear to have been fairly stable over the time period of interest whereas autism prevalence has steadily increased (Figure 2). A similar pattern is likely in the rest of the U.S. as well as in most other western nations.

With respect to the evaluation of the potential association of autism prevalence with emissions of other pollutants, we found most did not yield statistically significant associations with relative risk (RR) between 1.0 and 1.2. The exception was nickel, which yielded a RR of 1.71 (95% CI = 1.12–2.60). Furthermore, the emissions data for nickel were highly skewed, with data for one location constituting an outlier in the analysis. When the analysis was run excluding this particular data point, the RR for the association between TRI air nickel and autism increased to 3.59 (95% CI = 1.63–7.94), an association nearly as strong as that for autism and air mercury. Interestingly, Windham et al. (2006) also observed a relationship between air nickel and autism.

TABLE 1. Results of Analysis of Mercury Air Emissions and Autism Prevalence in Texas Counties and School Districts. Further detail provided in Lewandowski et al. (2009).

Academic year	TRI reporting year	RR Air Mercury and Autism (95% CI) [censored data treated as zero]		RR Air Mercury and Autism (95% CI) [censored data treated as midpoint of censored data range]	
2000–2001	2001	4.45	(1.6–12.4)	1.28	(0.95–1.72)
2001–2002	2002	2.7	(1.17–6.15)	1.51	(0.91–1.47)
2002–2003	2003	2.07	(0.84–5.13)	1.12	(0.83–1.50)
2003–2004	2004	1.41	(0.56–3.53)	1.06	(0.81–1.38)
2004–2005	2005	1.19	(0.54–2.61)	1.03	(0.81–1.31)
Analysis allowing for latency between exposure and case ascertainment					
2005–2006	2000	1.56	(0.36–6.87)	1.03	(0.59–1.83)
2006–2007	2001	2.49	(0.63–9.39)	0.94	(0.59–1.47)

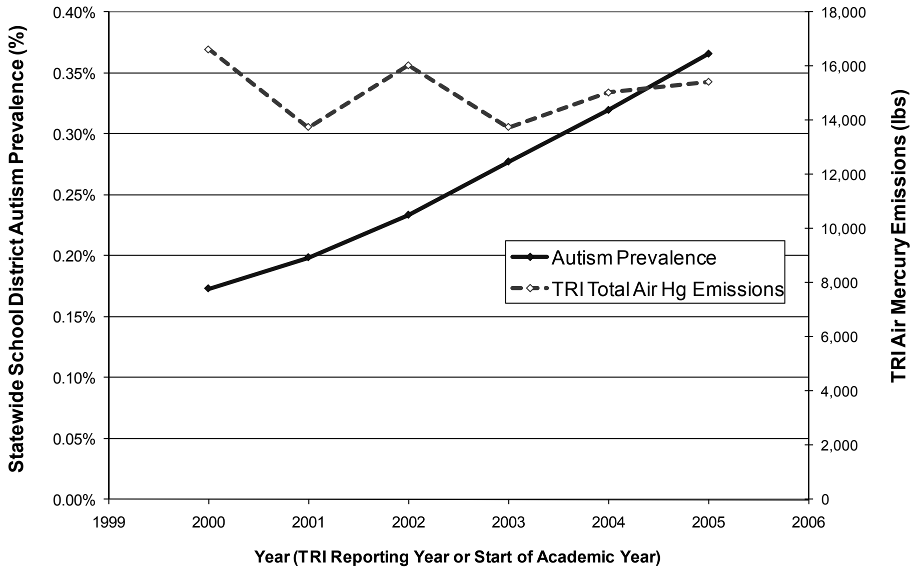

Figure 2. Summary of Mercury Air Emissions and Autism Prevalence in the U.S. State of Texas, 2000–2005. School district autism data were obtained from the Texas Education Agency. TRI air mercury data were obtained from the U.S. EPA (2010).

Evaluations using downwind location from coal-fired power plants or existence of mercury related fish consumption advisories as the surrogate exposure variables did not yield statistically significant results. For example, use of a weighted variable to characterize estimate mercury exposure also did not indicate a significant association between downwind status and autism prevalence (RR = 1.15, CI = 0.52–2.55).

It was surprising that the manner of handling censored data had such a highly significant effect on the analysis results; i.e., results which were statistically significant when censored data were handed as zeros (a common approach) became non-statistically significant when they were replaced with the midpoint of the possible censored value range. This demonstrates the extreme sensitivity of these types of ecological analyses to different methods of data handling and should serve as a caution to other researchers using state education data for this type of analysis.

5. Conclusions

Mercury is a neurodevelopmental toxicant whose anthropogenic emissions should be controlled. This is particularly important given anticipated increases in mercury emission rates in economically expanding parts of Asia. However, while methylmercury has been linked to subtle neurodevelopmental effects (i.e., cognitive test deficits detectable only on a population scale) the great majority of the evidence indicates that ethylmercury exposure via vaccines is not associated with the observed increased prevalence of autism. In addition, there are no data which clearly demonstrate that other forms of mercury (methylmercury, elemental mercury, inorganic mercury) are causal factors in autism. Several ecological studies which have suggested support for an association have serious limitations and the overall hypothesis is contradicted by the experience of studies conducted in populations with higher and better ascertained exposures where an increased prevalence of autism is not observed. What is now required is a well controlled case-control or cohort study with extensive pre- and post-natal mercury exposure assessment. Conducting such a study will not be easy, particularly if it attempts to address concerns about a yet-to-be defined sensitive subpopulation of children with autism. For that reason, government agencies, advocacy groups and mercury emitting industries should all consider supporting such studies (including those that may already be underway) so that this contentious health concern can be definitively addressed.

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HEARING CHANGES IN CHILDREN EXPOSED TO ARSENIC IN NEUROTOXICITY CONTEXT

VLADIMÍR BENCKO*
Institute of Hygiene and Epidemiology, First Faculty of Medicine
Charles University in Prague and General University Hospital
Studnickova 7, CZ 12800 Prague 2, Czech Republic

Abstract. Arsenic is element ubiquitously distributed in nature. Human exposure to this element is mediated by drinking water (e.g. in Bangladesh, Taiwan, Argentine, Chile, United States, Hungary), via local food grown in highly contaminated regions e.g. due to burning arsenic rich coal or in the vicinity of smelters, especially around copper and lead smelters and after a mass use of pesticides e.g. defoliant-dimethylarsinic acid- in a mass scale used by US troops in Vietnam as the Agent Blue or by see food items. In copper, lead and stibium smelters there are frequent chances for inhalation exposure which was described as well in connection with application of pesticides containing this metaloid. There were described as well iatrogenic and accidental intoxications with sever clinical consequences even with fatalities. The environmental and occupational toxicology of arsenic has been extensively reviewed (e.g. EPA, 1984; Clarkson, 2001; Bencko and Slámová, 2007; ATSDR, 2007). The limited extent and a specific orientation of this chapter citation of original papers is limited and served for documentation of highlights in the text or for illustration of unique human exposure situations.

Keywords: Arsenic toxicity, environmental toxicology, arsenic rich coal, coal burning, toxic damage to hearing, arsenic tolerance

1. Objective of the Study

Several epidemiological studies have been performed to determine the impact of environment on health in an area excessively polluted with arsenic-containing emissions in Upper Nitra Valey, Central Slovakia Region. Emissions

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* To whom correspondence should be addressed: *vladimir.bencko@lf1.cuni.cz*

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were produced by a power plant burning local arsenic rich coal. Despite the use of electrostatic eliminators, about half a ton of arsenic was emitted per day. The analysis of medical statistics gave evidence of a high rate of occurrence of skin and gastrointestinal diseases in the population living in the area. Clinical and hematological examinations on groups of 10-year-old children revealed warning changes in blood profiles and hemoglobin values, which frequently approached the extremes of normal physiological limits. The morbidity rate in children, from birth to the age of 15, has been semilongitudinally studied and the results have been compared with those obtained in suitably selected reference areas. The incidence rate of respiratory diseases as well as incidence rates of eye, skin, and subcutaneous tissue diseases was markedly higher in exposed children (Medvedová and Cmarko, 1974). We have found elevated concentrations of arsenic in the blood, urine, and hair of 10-year-old children in the area (Bencko and Symon, 1977).

During the collection of samples for the ecological study, the teachers of music education at a school situated about one km from the above-mentioned power plant voiced complaints about the singing ability of their pupils. This led to our undertaking a pilot study limited to audiometric examination of air conduction. After obtaining some interesting results, we compiled the following detailed study of the problem.

1.1. METHODS

Audiometric examination is a subjective method and the results can be influenced by the technique of measurement of the hearing thresholds, as well as by errors or negligence on the part of the person examined (degree of concentration, accuracy of reaction to given signals, and possibility of simulation or dissimulation). Our audiometric examination of the two exposed and reference groups of children were open to possible errors arising from the subjective character of the method. To minimize the potential influence of these errors the investigation was standardized as follows: All examinations were performed by one thoroughly trained person, using the same apparatus (portable performance Co. Kamplex TA 51), during the same time of day, 8:30–11:30 AM, in silent audiologic chambers. Both groups of children were approximately of the same size (exposed group 56, control 51 children), and the ratio of boys and girls was almost the same. The ages of the children averaged about 10 years, with limits of 9.5–11 years. From the above-mentioned, it follows that efforts were made not only to standardize technique of measurement, but also to examine, as far as possible, homogeneous groups of individuals.

Thresholds of hearing were examined at frequencies of 125–8,000 Hz for air conduction, and 125–4,000 Hz for bone conduction. Children with symptoms of acute nasopharyngitis were examined only after they had recovered from the condition or excluded from the study. Both groups of children were examined for symptoms of otorhinolaryngological disease throughout the course of audiometric testing. The status of the nasopharynx and of the vestibular apparatus was examined, and, besides otoscopic examination, and orientational examination of musical hearing was performed. Because clinical examination is itself to some extent subjective, both groups of children were examined by two experienced clinicians (Professor V. Chládek, M.D., Ph.D., and Professor J. Pihrt, M.D., Ph.D.), each of whom did his own examinations in both groups.

Audiometric and clinical examinations were complemented with a questionnaire analysis. Questionnaires were filled out by the parents of the children. The objectives of this part of our work were to detect any possible congenital causes of hearing impairment, maternal influenza or rubella during pregnancy, delivery trauma, or hereditary factors, and to identify children with somatic parameters not corresponding to their ages. We did not find any such case. The remaining questions were in connection with possible hearing damage due to infectious diseases, as well as present status of tonsils (i.e., removed or not), presence of middle ear inflammation, discharge from the ears, or frequent rhinitis in the personal medical histories of the children.

Data collected from these three sources on each child were evaluated statistically.

1.2. RESULTS

Differences between exposed and control group are demonstrated in Tables 1–3 and were tested by the χ^2 test (see Table 4). We also forego the use of “mean value” of the hearing threshold, which is only a statistical value. The hearing ability of each individual was evaluated at each frequency by putting measured values for the right and left ear on the X and Y axis, respectively. The plane of each graph (see Figure 1) was divided into parts, representing ranges of intensity, in 5-dB intervals. According to the presence in different ranges examined persons (at each frequency separately) were divided into classes and the scores of both exposed and reference groups in the classes mentioned above were used as test criteria for statistical evaluation. This procedure for group diagnosis is fully acceptable from the standpoint of statistics.

The reason we present the computed values from the χ^2 test is to help in detailed consideration of the P values. We found the same degree of importance as in our pilot study (Symon and Bencko, 1973) at frequencies of 125, 250, and 8,000 Hz in the case of air conduction. At a frequency of 500 Hz, a difference between group means was found only at the 5% level in both bases. This was also true for this series at the 1,000 Hz frequency, the hertz value being only 0.7152 under the critical value of 7.815. It can thus be concluded that, in the case of air conduction, important hearing losses were found at frequencies of 125, 250, and 800 Hz, especially at the lowest frequency range ($P < 0.0005$).

TABLE 1. Hearing Threshold (Mean Levels) at Frequencies Examined in Reference Group (51 Children) for air as well as bone conduction* (Quoted from Bencko and Symon (1977)).

Conduction and frequency	Boys (26) (Left/right ear)		Girls (25) (Left/right ear)		Mean value
Air					
125	6.54	7.69	8.20	8.80	7.80
250	4.42	5.00	5.00	6.00	5.10
500	5.38	6.73	5.00	7.20	6.08
1,000	3.27	3.85	2.60	6.40	4.02
2,000	5.96	3.27	5.80	3.40	4.61
4,000	7.31	6.15	4.80	4.20	5.64
8,000	14.42	12.69	10.40	13.20	12.70
Bone					
125	10.77	15.19	11.80	15.00	13.19
250	5.19	7.50	6.20	6.00	6.37
500	2.12	2.50	3.80	2.80	2.80
1,000	3.85	3.46	3.20	5.20	3.92
2,000	2.69	2.88	2.20	3.00	2.70
4,000	−7.31	−8.27	−9.00	−8.00	−7.79

* Values are given in decibels.

TABLE 2. Hearing Threshold (Mean Levels) at Frequency Ranges Examined in Exposed Group (56 Children) for Air and Bone Conduction* (Quoted from Bencko and Symon (1977)).

Conduction and frequency	Boys (26) (Left/right ear)		Girls (25) (Left/right ear)		Mean value
Air					
125	11.00	11.00	11.35	10.58	10.99
250	8.50	8.00	8.46	8.08	8.26
500	9.17	8.67	7.88	7.88	8.44
1,000	6.17	6.50	4.42	4.81	5.27
2,000	6.83	4.17	8.27	5.58	6.16
4,000	9.67	9.33	7.31	7.50	8.53
8,000	19.33	15.33	18.08	16.15	17.23
Bone					
125	16.17	17.00	19.04	20.00	17.95
250	8.83	13.83	8.65	11.15	10.67
500	4.67	7.67	7.31	6.35	6.48
1,000	5.00	6.50	4.62	5.77	5.49
2,000	1.17	3.17	3.27	3.08	2.64
4,000	−0.50	2.67	−1.15	−2.12	−1.61

* Values are given in decibels.

TABLE 3. Rate of Hearing Threshold in Different Ranges of Intensity (<5, 10, 15, and >20 dB) at the Frequency Examined Ranges* (Quoted from Bencko and Symon 1977).

Conduction and frequency	Intensity ranges (dB)							
	<5		10		15		>20	
	A	B	A	B	A	B	A	B
Air								
125	15	3	27	24	9	25	0	4
250	33	14	13	32	5	8	0	2
500	26	14	16	30	7	9	2	3
1,000	38	32	6	19	6	3	2	1
2,000	30	24	12	22	5	8	4	2
4,000	21	16	22	19	5	15	3	6
8,000	7	0	15	9	11	11	18	36
Bone								
125	2	0	14	5	20	11	15	40
250	23	6	23	24	5	19	0	7
500	39	26	10	19	2	10	0	1
1,000	32	28	14	17	4	10	1	1
2,000	34	38	10	13	6	5	1	0
4,000	49	42	1	10	1	2	0	2

* A = reference group; B = exposed group of 10-year-old children. Figures in the table indicate the numbers of individuals.

TABLE 4: Statistical Significance of the Differences Found Between the Hearing Thresholds of the Reference and Exposed Children at the Range of Frequencies Examined (Quoted from Bencko and Symon 1977).

Conduction and frequency	$\chi 2$ Value	*P*
Air		
125	23.3190	0.0005
250	18.2016	0.001
500	11.1076	0.05
1,000	8.5302	0.05
2,000	4.7435	–
4,000	6.2952	–
8,000	17.9612	0.01
Bone		
125	31.6470	0.0005
250	20.0987	0.001
500	11.5174	0.01
1,000	2.8975	–
2,000	1.4601	–
4,000	10.0048	0.05

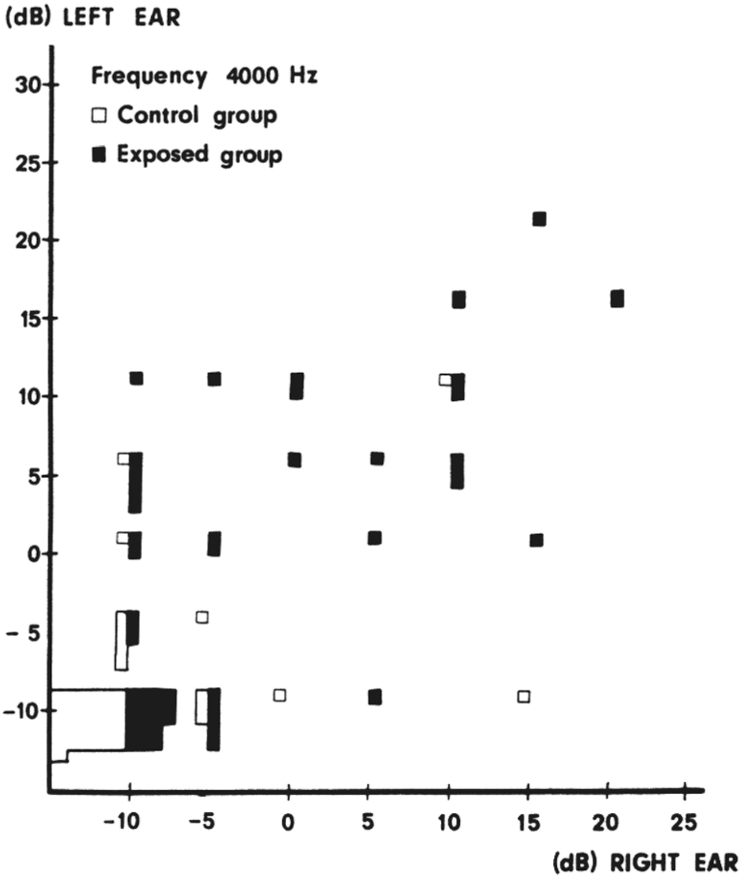

Figure 1. Hearing thresholds: Bone conduction (quoted from Bencko et al., 1977).

Significant degrees of hearing loss were found in bone conduction as well. The findings of greatest significance were at low frequencies *(P* < 0.01); at high frequencies, a level of significance of 5% was found only at 4000 Hz. A very simple examination of musical hearing sense (see Table 5) was based on the child's reproduction of a "very well known song" (anthem), and on his ability to mark time with music (tapping with a pencil). Vestibular examination (see Table 6) showed that at the age of about 10 years fit is impossible to judge results of cupulometric examination because the reactivity of the vestibular apparatus depends upon the current motor activity of children. It may explain the fact that in exposed children results comparable to those of adults were found. Children living in the country, with a greater possibility of free movement, are more developed in comparison to control, city residents, who are markedly handicapped in this respect. It pays, without any change, for the examination of caloric nystagmus and high overthreshold irritation. It is reflected especially in the results of walking backward with eyes closed. This simple examination seems to be very suitable for testing the degree of motor development in schoolchildren and adolescents.

TABLE 5. Results of Orientational Examinations to Determine: (a) Ability to Reproduce Given Musical Sounds (Tones); (b) Ability to Tap in Time to Musical Rhythm; (c) Marks Achieved in Music Education* (Quoted from Bencko and Symon 1977).

	Reference children	Exposed children
Reproduction of tones		
Good	46.5	37.2
Mean	34.1	40.5
Bad	19.4	21.3
Rhythm disorders		
None	63.3	62.7
Little	21.9	31.1
Great	14.8	6.2
Mark from music education		
(1) Excellent	71.0	65.0
(2) Very good	22.0	28.8
(3) Good	7.0	6.2

* Figures represent percentages of each group.

1.3. DICUSSION

The statistical significance of the hearing impairments found points to auditory damage, including total deafness, caused by arsenic poisoning in clinical experience (Beck, 1926; Yamashita et al., 1972) and damage to inner ear has been repeatedly proved in animal experiments as well (e.g.Westerhagen, 1970; Anniko, 1976). But still the possibility of supracochlear damage cannot yet be excluded.

TABLE 6. Results of Clinical Examination of Reference (A) and Exposed (B) Children* (Quoted from Bencko and Symon 1977).

	A		B	
	N	%	*N*	%
Normal otosopic finding				
Yes	51	(100.00)	56	(100.00)
No	–		–	
Tubal disorder				
Yes	–		1	(1.78)
No	51	(100.00)	55	(98.22)

(*continued*)

(*continued TABLE 6.*)

	A		B	
	N	*%*	*N*	*%*
Tonsils				
Normal	37	(72.55)	31	(55.36)
Enlarged	3	(5.88)	12	(21.43)
Large	3	(5.88)	9	(16.07)
Status post TE	8	(15.69)	4	(7.14)
Adenoid vegetations				
Normal	40	(78.43)	31	(55.36)
Enlarged	7	(13.73)	13	(23.21)
Large	4	(7.84)	12	(21.43)
Mucopurulent secreta flowage down the dorsal part of nasopharynx				
Yes	5	(9.80)	13	(23.21)
No	46	(90.20)	43	(76.79)
Tinnitus				
Yes	–		1	(1.78)
No	51	(100.00)	55	(98.22)
Irritability of vestibular apparatus				
Normal	25	(49.02)	21	(37.50)
Increased	22	(43.14)	34	(60.71)
Asymmetric	4	(7.84)	1	(1.78)
Threshold of irritation				
Normal	17	(33.33)	31	(55.36)
Increased	33	(64.71)	24	(42.86)
Asymmetric	1	(1.96)	1	(1.78)
Divergences of arms				
Normal finding	8	(15.69)	7	(12.50)
Minimal	41	(80.39)	46	(82.14)
Large	2	(3.92)	3	(5.36)
Slight decrease of arms				
Yes	10	(19.61)	3	(5.36)
No	41	(80.39)	53	(94.64)
Walking backward with eyes closed				
Normal	26	(50.98)	42	(75.00)
Abnormal	25	(49.02)	14	(25.00)
Walking forward with eyes closed				
Normal	45	(88.24)	49	(87.50)
Abnormal	6	(11.76)	7	(12.50)
Caloric nystagmus				
Normal	31	(60.78)	45	(80.36)
Decreased	15	(29.41)	6	(10.71)
Almost absent	4	(7.84)	4	(7.14)
Asymmetric	1	(1.96)	1	(1.78)
Fixational nystagmus				
Yes	6	(11.76)	4	(7.14)
No	45	(88.24)	52	(92.86)

* Control = 51, exposed group = 56 individuals.

There exists no uniform rule on age limits for the use of subjective methods for measuring hearing threshold. Generally an inverted relation is stated between low age and the reliability of results obtained. Different authors give age limits ranging from 3 to 7 years (Mylkebust 1960). According to clinical experience, the use of audiometry for evaluation of both air and bone conductive hearing in children aged 10 (such as composed our groups) is fully acceptable. Because of transmission difficulties which we expected after the first series of examinations, the person examined responds with less accuracy. For this reason we not only standardized the examination procedure (see above), but performed the audiometric examinations with the least possible stress to the children. There was no rushing about and more than 25 min was allotted for the examination of each child. To avoid any potential objections concerning a diurnal fluctuation of hearing sensitivity we performed all audiometric examinations between 8:30 and 11:30 AM. Hearing losses could be explained as a result of scarring caused by mitigated infections surrounding the Eustachian tubes and compromising their ventilatory function; did we interpret results of the first series of examinations. In exposed children, the rhinophonia clausa can be expected, resulting in defects as well as losses of bone conduction left no grounds for the previous simple interpretation. The group more frequently suffered from inflammation of the middle ear (there had been an epidemic of scarlet fever in this class several years ago). While an assumed influence of the anamnestic presence of middle ear inflammation is fully documented in the reference group (see Table 7), such does not appear to be the case in the exposed group. The character of the losses encountered suggests possible toxic supracochlear damage.

TABLE 7. Results of Questionnaire Analysis (Anamnestic Data on Children Examined)* (Quoted from Bencko and Symon 1977).

	Reference group		Exposed group	
	N	%	*N*	%
Measles	35	(68.63)	42	(75.00)
Scarlet fever	12	(23.53)	3	(5.36)
Mumps	22	(43.14)	38	(67.86)
Meningitis epidemica	2	(3.92)	2	(3.57)
Frequent rhinitis	11	(21.57)	22	(39.29)
Adenoid vegetations removed	13	(25.49)	3	(5.36)
Middle ear inflammations	16	(31.37)	8	(14.29)
Discharge from ears	3	(5.88)	2	(3.57)
Rubella or serious influenza during pregnancy	–		3	(5.36)
Musical instrument players	6	(11.76)	9	(16.07)

* The first column lists diseases for which the numbers of occurrences in and percentages of each group are given.

2. Neurotoxicity Context

Acute, high-dose exposures (2 mg As/kg/day or above) often lead to encephalopathy, with signs and symptoms such as headache, lethargy, mental confusion, hallucination, seizures, and coma (e.g. Uede and Furukawa 2003; Vantroyen et al., 2004). The similarity of the clinical symptoms of arsenical encelopathy (hemorrhagic encephalitis), once the much-feared complication of iatrogenic arsenic intoxication with Wernicke's syndrome or Korsacoff's psychosis was stated in the literature (Bencko 1987). Considering the biochemical nature of these syndromes, such a clinical resemblance is not surprising. The cause of both syndromes lies in the deficit of thiamine, the cofactor of pyruvic acid decarboxylase. Arsenic is known to block lipoic acid, which is another cofactor of the same reaction.

Repeated exposures to lower levels (0.03–0.1 mg As/kg/day) are typically characterized by a symmetrical peripheral neuropathy (e.g. Buchancová et al., 1988, Muzi et al., 2001, Bencko and Slámová, 2007). This neuropathy usually begins as numbness in the hands and feet, but later may develop into a painful "pins and needles" sensation. Both sensory and motor nerves are affected, and muscle weakness often develops, sometimes leading to wrist-drop or ankle-drop. Diminished sensitivity to stimulation and abnormal patellar reflexes has also been reported. Numbness and tingling of the finger tips, toes, and circumoral region were reported by a women exposed to an unspecified amount of arsenic in bird's nest soup. Discontinuation of exposure resulted in the disappearance of symptoms (Luong and Nguyen, 1999).

Histological examination of nerves from affected individuals reveals a dying-back axonopathy with demyelination (Goebel et al., 1990). Some recovery may occur following cessation of exposure, but this is a slow process and recovery is usually incomplete. Peripheral neuropathy is also sometimes seen following acute highdose exposures, with or without the previously described encephalopathy (Baker et al., 2005; Kamijo et al., 1998).

Neurological effects were not generally found in populations chronically exposed to doses of 0.006 mg As/kg/day or less (EPA 1984), although fatigue, headache, dizziness, insomnia, nightmare, and numbness of the extremities were among the symptoms reported at 0.005, but not 0.004 mg As/kg/day in a study of 31,141 inhabitants of 77 villages in Xinjiang, China (Lianfang and Jianzhong 1994), and depression was reported in some Wisconsin residents exposed to 2–10 μg As/L in the drinking water for 20 years or longer (Zierold et al., 2004).

2.1. INTELLECTUAL FUNCTIONS RELATED ISSUESS

There is emerging evidence suggesting that exposure to arsenic may be associated with intellectual deficits in children which was comprehensively and critically reviewed by ATSDR (2007). Wasserman et al. (2004) conducted a cross-sectional evaluation of intellectual function in 201 children 10 years of age whose parents were part of a larger cohort in Bangladesh. Intellectual function was measured using tests drawn from the Wechsler Intelligence Scale for Children; results were assessed by summing related items into Verbal, Performance, and Full-Scale raw scores. The mean arsenic concentration in the water was 0.118 mg/L. After adjustment for confounding factors, a dose-related inverse effect of arsenic exposure was seen on both Performance and Full-Scale subset scores; for both end points, exposure to ≥50 μg/L resulted in statistically significant differences ($P < 0.05$) relative to the lowest exposure group (<5.5 μg/L). In a later report, the same group of investigators examined 301 6-year-old children from the same area (Wasserman et al. 2007). In this case, the children were categorized based on water arsenic concentration. After adjustment for water Mn, blood lead, and socio-demographic features known to contribute to intellectual function, water arsenic was significantly negatively associated with both Performance and Processing speed raw scores. Analyses of the dose-response showed that compared to the first quartile, those in the second and third categories had significantly lower Performance raw scores ($P < 0.03$ and $P = 0.05$, respectively). Those in the fourth category had marginally significantly lower Full-Scale and Processing Speed raw scores. It should be mentioned, however, that in general, arsenic in the water explained <1% of the variance in test scores. Water arsenic made no contribution to IQ outcomes. A study of 351 children age 5–15 years from West Bengal, India, found significant associations between urinary arsenic concentrations and reductions in scores of vocabulary tests, object assembly, and picture completion; the magnitude of the reductions varied between 12 and 21% (von Ehrenstein et al. 2007). In this cohort, the average lifetime peak arsenic concentration in well water was 0.147 mg/L. However, no clear pattern was found for increasing categories of peak arsenic water concentrations since birth and children's scores in the various neurobehavioral tests conducted. Furthermore, using peak arsenic as a continuous variable in the regression models also did not support an adverse effect on the tests results. Exposure to arsenic in utero also did not suggest an association with the tests scores. Von Ehrestein et al. (2007) concluded that the study provided little evidence for an effect of long-term arsenic concentrations in drinking water and that the lack of findings with past exposures via drinking water may be due to incomplete assessment of past exposure, particularly exposure originating from food. Wasserman's results are consistent with those of ecological studies in children in Taiwan (Tsai et al. 2003) and in China (Wang et al. 2007). In the former, adolescents exposed to low (0.0017–0.0018 mg As/kg/day) levels of inorganic arsenic in the drinking water showed decreased performance in the switching attention task, while children in the high exposure group (0.0034–0.0042 mg

As/kg/day) showed decreased performance in both the switching attention task and in tests of pattern memory, relative to unexposed controls. In the study in China children whose mean arsenic concentration in the drinking water was 0.190 mg/L had a mean IQ score of 95 compared with 101 for children with 0.142 mg/L arsenic in the water and 105 for control children with 0.002 mg/L arsenic in the drinking water (Wang et al. 2007). The differences in IQ scores between the two exposure groups and the control group were statistically significant. Nevertheless no other potential risk factors and socio-demographic features contributing to intellectual functions were taken into consideration in this study.

2.2. HISTORY, PRESENT STATE & FUTURE RESEARCH CHALLENGES

Ehrlich's attempt to interpret the mechanism of arsenic toxicity on the *Treponema pallidum* in chemical terms as its interaction with the side chains of protoplasmatic proteins (Antman, 2001) was historically the first one ever made, yet further research work proved to be relevant. During World War II a great deal of research interest was centered on the arsenic-sulfhydryl group interaction. In most cases the reason for this intensive research work was the potential risk of the use of lewisite. Stimulated by a similar concern, such studies aimed at anti-lewisite protection had already appeared as well in prewar Czechoslovakia (e.g., Roubal and Pokorný, 1939). These researches brought in a great number of enzymes sensitive to the action of arsenic (Barron et al., 1947). It became obvious that the protective effect of various thiolo-substances against the toxic activity of arsenic is far from uniform. The research showed that some of proteins tend to react with lewisite by binding to it with two SH groups, forming a ring that is not freely reversible by the action of monothio substances. These findings prompted further research into the activity of dithio substances. As a result, 2,3-dimercaptopropanol (DMP, British Anti-Lewisite) was discovered; this turned out to be effective both under *in vivo* and *in vitro* conditions. The specific role played by DMP in the protection of the pyruvic acid oxidase system aroused a suggestion that it might be some kind of cyclic dithiol that acts as a cofactor in these reactions. This cofactor was later identified as the lipoic acid, an aliphatic dithiol. It is assumed that the protective mechanism of DMP lies in that the 6-member lipoate-arsenate ring is less stable than the 5-member dimercaptopropanol-arsenate ring, which by competition results in the binding of arsenic to DMP. The protective effect of thio substances (e.g., glutathione and cysteine) against the toxic action of trivalent arsenic is a proof that one of the alternative mechanisms of the arsenic toxicity lies in an evidently reversible bond with the sulfhydryl groups of proteins. A high-degree of sensitivity to trivalent arsenic was found in the enzymes urease and choline-dehydrogenase. Little sensitivity is exhibited by invertase, catalase and cytochrome oxidase. In strictly anaerobic *Cl. sporogenes* it inhibited the activity of glycine and alanine desaminases. The inhibitory effect of arsenates on the potato phosphatase activity is 50 times the effect of phosphates. In rat skin tissue cultures they markedly suppressed the cholesterol biosynthesis. This action is in association with the cumulation of lanosterol and represents a radical intervention into the biosynthesis of skin sterols. Apart from blocking the SH groups of proteins, which is biologically most important in case of thiolo-enzymes, especially hydrolases, arsenic may also react, through some other mechanisms, with the biological systems. As a competitive inhibitor of phosphates, arsenate may compete with them for the binding sites on a number of enzymes, especially in the system of phosphoraldehyde and glucose dehydrogenase. The arsenic for phosphate substitution in this system can be ascribed to the fact that arsenate esters are much easier hydrolyzed than the relatively stable phosphate esters. This phenomenon called “arsenolysis” was confined experimentally using radioactive isotopes and is employed to explain the enhanced activity of various enzyme systems which can be commonly observed in the presence of arsenate ions. The fact that arsenites may have a deep impact on oxidative phosphorylation can be explained analogously. The unstable arsenylated oxidation product is subjected to irreversible hydrolysis which leads to enhanced oxidation of the substrate, but without the formation of high-energy phosphate bonds, which is energetically less favorable.

2.3. ARSENIC TOLERANCE

In the case of arsenic, the tolerance dose as well as the latency period may greatly vary from individual to individual. Oftentimes it takes months, or even years, for the appearance of the first clinical symptoms of chronic arsenic poisoning. It is assumed that a permanent intake of about 10 mg of arsenic a day suffices to produce the symptomatology of chronic arsenic intoxication. However, some individuals can tolerate as many as 20 mg arsenic three times a day, ingested for an apparently long time period, without showing any clinical signs of poisoning. A specific example in this context is the category of the habitual “eaters” of arsenic (arsenicophagy), who are referred to in most toxicological and pharmacological textbooks. These individuals took appreciable doses of arsenic regularly, commonly once or twice a week, allegedly as a roborant or tonic. The doses taken ranged widely, but the most common intake pattern was 40 mg arsenic twice a week. The assumption is that this habitual abuse of arsenic is not of protoplasmic nature, for its discontinuation is not known to produce any irresistible compulsion to continue the use of arsenic. Most authors agreed that the harm-free ingestion of such overdoses of arsenic is a result of reduced arsenic uptakes from the gastrointestinal tract (known gradual increase of the therapeutic dose in clinical practice of a historical terapeutic use of arsenicals like *Fowler’s solution* or *Asiatic pills*), and the general belief is that no rise in tolerance for parenteral doses of arsenic can be expected in these individuals. For clearly understandable reasons, this hypothesis could not have been confirmed in man. In our experiments with mice we observed a marked rise in the LD50 value for an i.m. (i.e., parenteral) administration of arsenic given to animals after their oral exposure to arsenic in drinking water, and an increased rate of arsenic excretion in animals previously exposed in the same manner. The acceleration of arsenic excretion rates can be partially ascribed to the accelerated biotransformation of the more toxic trivalent arsenic to its less toxic pentavalent form, and to the fact that a substantial portion of the arsenic administered is rapidy alkylated and excreted as a mono and dimethyl-derivatives (e.g. Crecelius, 1977; Bencko et al.,1976). Shares of these two reactions are not constant and vary from one animal species to another (Vahter and Norin, 1980). Arsenic is reported to markedly decrease the metabolic consumption of oxygen. A tempting and, perhaps, quite a rational idea is that it could be this reduction of tissue breathing, resulting in the lowering of general oxygen demand which, many years ago, prompted some smugglers to use overdoses of arsenic to help them get over the hard-to-cross passes and ridges of the Alps mountains.

3. Conclusions

Present research interest concentrate on alteration of cellular signals transduction, activation of transcription factors, changes in gene expression, promotion of cell proliferation, differentiation, and apoptosis leading to cell death, or cancer development mediated by the production of reactive oxygen species induced by arsenic exposure (Florea et al. 2005). The wide spectrum of its mechanisms of toxicity substantiate its importance among xenobiotics that are of a high public health concern. In this context there is a growing sense of urgency to develop a biologically based dose response models of health risk assessment, integrating emerging data from molecular biology and epidemiology to provide more realistic data for risk assessors, public health managers and environmental issues administrators.

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LEAD OCCURRENCE IN CHILDREN'S BIOLOGICAL FLUIDS FROM BAIA MARE AREA, ROMANIA

VASILE OROS[1]*, SIMONA ROMAN[2] AND MIRELA COMAN[1] AND ANCA DIANA OROS[3]
[1] *Department of Mineral Processing, Geology and Environment Engineering, North University Baia Mare*
62A Dr. Victor Babes Str., Baia Mare, Romania
[2] *The Public Health Authorithy of Maramures County*
31 G. Cosbuc Str., Baia Mare, Romania
[3] *Child and Adolescent Psychiatry Department*
Obregia Hospital of Psychiatry
10 Berceni Str, Sector 4, Bucharest, Romania

Abstract. Baia Mare is a municipality in northwestern Romania and the main city of Maramures County. As it is located in a region having a lot of non-ferrous metals and precious metals deposits, an intensive activity of mining and metallurgy has been developed. The municipality of Baia Mare currently has a total population of 137,976 of which some 7,128 peoples are living in Ferneziu area, situated in the northwest of the city. This population is the most exposed to high risks of current or historical heavy metal pollution because it is living within the very closed area around the metallurgical factory of lead smelter. This paper will deal with the actual and historical pollution with heavy metals and with the health problems caused by current air pollution and risks related to historical soil pollution. The most important risk sources for the people of Baia Mare are current air pollution and soil contamination resulted from historical pollution from two large metallurgical factories located within the town. The lead level in blood of children has drastically diminished during the last 20 years but there exists still about 40% of children having values over 10 μg/100 mL that indicate lead exposure and potential health risk. A special issue is registered in Ferneziu area which needs a special investigation.

Keywords: Lead, pollution, health, blood, children

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* To whom correspondence should be addressed: orosubm@gmail.com

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1. Introduction

Baia Mare is a municipality in northwestern Romania and the main city of Maramures County. Baia Mare city is emplaced at the border of Gutai Mts. and Baia Mare depression (Figures 1 and 2). As it is located in a region having a lot of non-ferrous metals and precious metals deposits, an intensive activity of mining and metallurgy has been developed. It has been known for a long time as a mining area; the gold-silver and base metal ore deposits have been mined for hundreds of years leading to the economic increase of the area and to the development of subsidiary activities related to ore processing.

During 1990' nine mines have being in exploitation around the town as well as two large mineral processing plants and two large metallurgical factories of Cu, Pb, Zn and Au, Ag were working quite in the town.

During the last 10 years the economic feature of the town has dramatically changed. All the mines have been closed (the last ones in 2007). In 2009 the largest metallurgical smelter (CUPROM – former Phoenix, producing mostly copper) has closed after more than 80 years of activity.

The activity of the second smelter (ROMPLUMB – producing mostly lead) is significantly diminished. At present it is within a transition period of technological improvement in the aim to become conforme with the environmental regulation limits (up to the end of 2010).

The former mining activities, mineral processing activities and metallurgical activities have outrun a lot of areas presenting intensive historical pollution with heavy metals. The impact of these activities on the environment are well-known: Baia Mare is a very polluted city and many efforts are joined now in order to remediate the area or minimize the heavy metal exposure of human population.

The municipality of Baia Mare currently has a total population of 137,976 of which 66,070 males and 71,906 females. Some 7,128 peoples are living in Ferneziu area, situated in the northwest of the city. This population is the most exposed to high risks of current or historical heavy metal pollution. This paper will deal with health problems caused by current air pollution and risks related to historical soil pollution. We will especially be concerned in lead pollution and particularly in health-realated risks in the case of children, who are known to be more sensitive to the effects of lead on health.

The most important risk sources for the people of Baia Mare are current air pollution and soil contamination resulted from historical pollution from two large metallurgical factories located within the town.

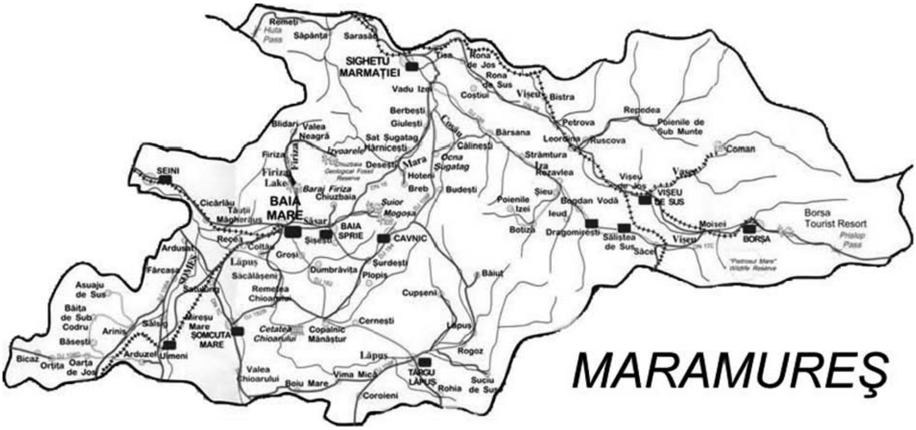


Figure 1. Geographical location of the Baia Mare area.

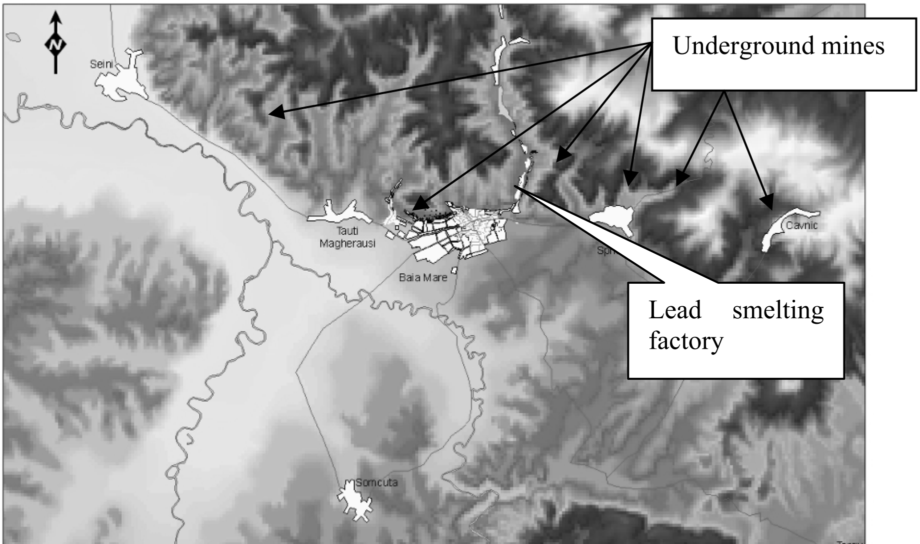


Figure 2. Location of the Baia Mare mining area.

2. The Feature of Heavy Metals Pollution in Baia Mare

2.1. THE MAIN SOURCES OF ENVIRONMENTAL POLLUTION IN BAIA MARE AREA

Actually the main sources of heavy metals environmental pollution are the former mining and mineral processing sites and the actual and former metallurgical factories. Moreover the pollution from transportation can be noticed as well as directly by gases and indirectly by the re-suspended dust containing heavy metals. In Table 1 the main sources of heavy metal pollution and risk of human exposure are summarized.

TABLE 1. The main sources of environmental pollution with heavy metals in Baia Mare.

Type of activity	Source of pollution	Ways of exposure and health risk	Receptors	Comments
Mining	Mining waste heaps	Oxidizing and leaching of heavy metals	Surface water Underground water	There exist more than 40 items of smaller or larger dams of mining wastes around the town Baia Mare
		Erosion	Air quality Land stability	
	Former mining galleries	Mining waste water	Surface water	There are 6 stations for treatment of the mining waste waters around Baia Mare
Mineral processing	Flotation tailing dams or ponds	Erosion	Air quality Land stability Soil pollution	Four large dams of flotation tailings
	Former flotation plants	Demolition	Degraded sites	Two degraded sites of flotation plants
Nonferrous metallurgy	The copper smelter factory CUPROM	Actually closed Historically polluted soil	Degraded sites	The high chimney (360 m) and the soil around the factory is polluted with heavy metals and As
	The lead smelter factory ROMPLUMB	Air pollution	Humans Soil	The soil in Ferneziu area is excessively polluted with lead and other metals
		Historically polluted soil	Humans Animals Vegetation	
Transportation	Vehicles	Gases from burning of fuel	Humans	The dust from the roads in Baia Mare is contaminated with heavy metals
		Re-suspended powder	Humans Soil	

On the photos of Figure 3 below are presented several sources of pollution with heavy metals around Baia Mare. a: mining waste waters from Ilba; b and c: mining waste dump from Ilba; d: mining waste dump from Suior; e: flotation tailing pond from Bozanta.

2.2. AIR POLLUTION

The two metallurgical factories ROMPLUMB and PHOENIX have been for a long time the major sources of air pollution with heavy metals and sulphur dioxide in Baia Mare. By roasting and smelting concentrates of metals sulphides, extremely small particles of metals and metal oxides are produced and evicted by through the chimneys. As a result, during a long period the air of Baia Mare has been polluted with heavy metals (Pb, Cu, Zn, Cd), arsenium and sulphur dioxide. In the aim of a better dispersion of pollutants a very high chimney (356 m) was built at the cooper smelting plant in Baia Mare in 1995–1998.

Another major source of air pollution is from the transport. The pollution of air because of the auto-transportation has the property of being "distributed" in the whole town as the auto-exhaust gasses are evacuated at the ground level, thus people are not able to avoid the polluting factors: organic volatile compounds, nitrogen oxides, sulphur dioxide, lead. The re-suspended dust from the roads in Baia Mare is also contaminated with heavy metals.

(a)

(b,c)

(d)

(e)

Figure 3. Sources of pollution with heavy metals around Baia Mare. (**a**) mining waste waters from Ilba; (**b**) and (**c**) mining waste dump from Ilba; (**d**) mining waste dump from Suior; (**e**) flotation tailing pond from Bozanta.

An evolution of the air pollution with lead in Baia Mare during 1989–2008 is presented as yearly average values in Figure 4.

Air pollution with lead has been very high during 1980–1990, the accepted levels (0.7 μg/m^3 average of 24 h has been the accepted level for that period) of lead content being exceeded with a frequency of 75% in 1981 and 96% in 1989 (Oros, 1993). A decrease in the air pollution with lead from 13.1 to 1.87 μg/m^3 yearly average for 1989 and 1998 respectively, has been registered during the period 1989–1998. Although the accepted level has remained constantly

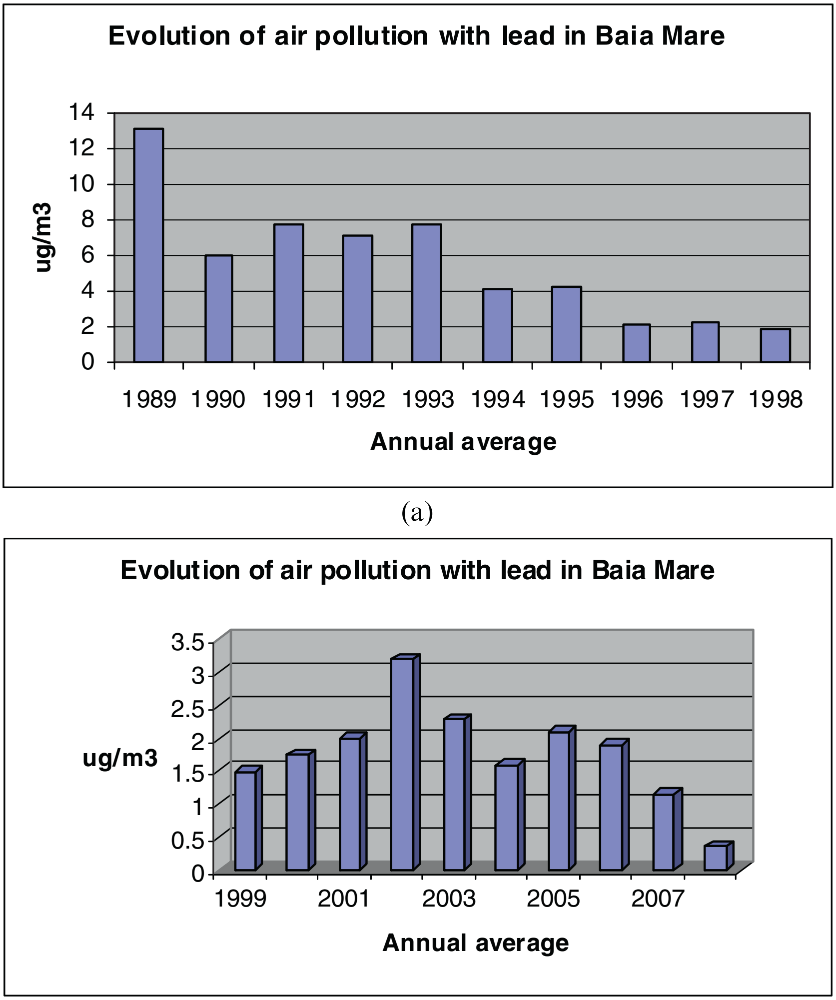


Figure 4. The evolution of air pollution in Baia Mare during 1989–1998 (**a**) and 1999–2008 (**b**).

exceeded, the frequency of exceeding percentage for the accepted daily average value has been 75–76% for 1997–1998 (Pop et al., 2002). Only in 2008 the yearly average for the air pollution with lead has registered a value below the accepted level (0.5 μg/m^3 the new accepted value).

Begining from 2008 the local Environmental Protection Agency from Maramures county (APMMM, 2010) is achieving automatically monitoring of the air quality in five monitoring stations. Two stations are located for the urban pollution, the third one for the suburban environment and the other two are located in Ferneziu area for monitoring the industrial pollution. The air pollution with lead is determined from powder suspention PM_{10} because the lead is evicted in the athmosfere as very fine particles. Table 2 summarised the usually mesured pollutants and the limits of the admited values.

The results reported for the period 2008–2010 (APMMM, 2008–2010) found that the air is polluted because of the traffic in the center area of the town and in Ferneziu area as result of the activity of lead smelter ROMPLUMB. For the urban area the results indicate a lead content of 0.25 μg/m^3, and it is 1.5 folder that the regional area. For the Ferneziu area the industrial pollution results in an average value of 0.83 μg/m^3. As conclusion of the reports of APMMM the lead smelter ROMPLUMB rest the maojor source of lead pollution of the air in Baia Mare despite the company has set efficient filters that capture the powder from the gases in the last period. Also a new equipment for remove sulphur dioxide from the gases has to be installed this year.

TABLE 2. The measured pollutants and the limits of admited values.

Pollutant	Period of average calculation	Admited limit value
Powder suspention (10 μm)	24 h (daily average)	50 μg/m^3 (not admited more than 35 values above registerd over a year)
	1 year (yearly average)	40 μg/m^3
Sulfur dioxide	1 h (hourly average)	350 μg/m^3
	24 h (dayly average)	125 μg/m^3 (not admited more than 24 values above registerd over a year)
Lead from powder suspention (10 μm)	1 year (yearly average)	0.5 μg/m^3

The air in Baia Mare has been polluted also with sulphur dioxide for a long period. A maximum value of yearly average of 251 μg/m^3 was registered in 1985 (Pop et al., 2002). After 1998 an important diminish has been registered to values below the accepted level of 60 μg/m^3. However, the previous periods when the accepted value was often exceeded have caused an important effect on the health of the population, but also on the environment. Some epidemiological researches showed an increased morbidity in Baia Mare in comparison with other regions that are not polluted (Oros et al., 2008). Respiratory illness were more frequently met here, the acute ones (150‰ in comparison with 104‰) and the chronic ones (22.4‰ compared to 6.7‰).

2.3. THE SOIL POLLUTION

The soil is a cumulative receptor of the heavy metals pollution. A part of the metals falling from the atmosphere in the soil is fixed on the organic phase or mineral phase of the soil. Obviously this thing leads to the accumulation of the heavy metals in the soil. Some studies that were made in this respect during 1980's suggest a soil pollution gradient with maximum concentrations that appears near the two smelting factories and a decrease of the pollution in a direct relation with the increase of the distance from the polluting source, following the direction of the wind to a distance up to 25 km. The majority of the polluting metals (some 70%) fall on a 7 km distance. Measuring in depth, the majority of the polluting agents can be found in superficial level, between 0 and 10 cm (Moldovan and Butnaru, 1991). They reported the following statement of the soil pollution with heavy metals:

The pollution with lead. It was approximated at over 30,000 ha of soil polluted with lead, that could be classified in three zones with different degree of pollution, as follows:

- almost 3,000 ha with a lead content over 1,500 ppm, considered as excessively polluted;
- over 10,000 ha with a lead content between 301 and 1,500 ppm, considered as very polluted;
- almost 18,000 ha with a lead content between 101 and 300 ppm, considered as moderately polluted

For the area around the city they reported also 31,000 ha with a lead content between 21 and 100 ppm (the maximal accepted level was being 100 ppm during that period) which represent a potential risk of pollution.

The pollution with zinc. It was approximated at almost 12,000 ha having a zinc content which exceeds 300 ppm, which was being the maximal accepted level. Some 6,500 ha from this surface has zinc content over 500 ppm and are considered as very polluted area. Some others 5,500 ha have zinc content between 301 and 500 ppm and are considered as moderately polluted.

The pollution with copper. It was approximated at over than 10,000 ha that have a copper content that exceeds 100 ppm. Some 4,500 ha of this surface has a copper content of over 300 ppm and it is considered as very polluted and 5,500 ha has a copper content of 101–300 ppm and it is considered as moderately polluted.

The pollution with cadmium. It was approximated at almost 6,000 ha that are polluted with cadmium and they are considered as moderately polluted with a content between 3.1and 10 ppm, the maximal accepted level being 3 ppm.

Other researches that have been done lately diminished the polluted area to a total of 27,000 ha and the excessively polluted zone to 1,700 ha (Pop et al., 2002). These researches consider that in comparison with 1987 a decrease with 15–60% of the metal concentration in the soil can be observed for copper. A decrease with 40–60% of the concentration for cadmium and a decrease of 25–30% of the concentration for zinc can also be noticed. However, an increase with 25–30% of the concentration for lead can be observed, with the exception of a few areas where the concentration decreases.

One can notice that besides the fact that there are large areas of polluted soil, the majority of these surfaces are polluted with Pb, Zn, Cu and Cd in the same time. As a tendency we can notice a decrease in time of the pollution with Zn, Cu and Cd that can be explained by the leaching and the self purification of the soil, sustained also by the decrease in the atmospheric pollution in the last period. For the lead, no such tendency can be observed, the fact being explained by the stronger fixation of this metal on the solid phase of the soil and by the pollution that is still produced by ROMPLUMB. A map of the soil pollution with lead in Baia Mare achieved by APMMM is presented on Figure 5. An excessive pollution is observed for the Ferneziu area (North West Baia Mare).

Figure 5. The map of the soil pollution with lead in Baia Mare (APMMM, 1998).

A study of the soil pollution of the Ferneziu area was achieved by Fülöp and Văscan in 2004. Analyses made on systematical samples taken off from 5 to 30 cm depth indicate extremely high contents of heavy metals. By processing their data on computer using Surfer 0.7, two maps have been achieved for each metal contents. Concerning the lead contents in the soil a map for the 5 cm depth is presented in Figure 6. The map shows that the highest concentrations of lead (over 3,500 ppm) in surface soil are located around the lead smelting plant and the old chimney. The medium concentrations class is specific for most of the area, the contents between 1,500 and 3,500 ppm of lead in surface soil is predominant in Ferneziu area.

The feature of distribution of lead concentration in 30 cm depth soil is different. The highest concentration class are much less extended and are located in two restricted areas eastern from the old chimney. Another area with strong pollution is located South-East towards Baia Mare city. The medium concentration classes of depth soil pollution is located in West area from plant and chimney (Fülöp and Văscan, 2004).

The Ferneziu area is very populated, houses with gardens and courthyards without green cover are present very closed around the smelting plant. Gardens containing very contaminated soils (more than 3,500 ppm) are used for growing vegetabels and fruits consumed within house.

The Romanian reglemented references for the lead content in soil are as follow:

- For the soil of sensitive use:
  - alert treshold: 50 mg/kg
  - intervention treshold: 100 mg/kg
- For the soil of less sensitive use:
  - alert treshold: 250 mg/kg
  - intervention treshold: 1,000 mg/kg

As cocnclusion of this study, the soil remains the most important source of lead for humans in Ferneziu area. The lead concentrations in soil at bouth depths are usually between 1,500 and 3,500 ppm and in some areas more than 3,500 ppm.

The recorded values exceed the threshold concentration limits reglemented for both sensitive and less sensitive soil uses, with very few exceptions.

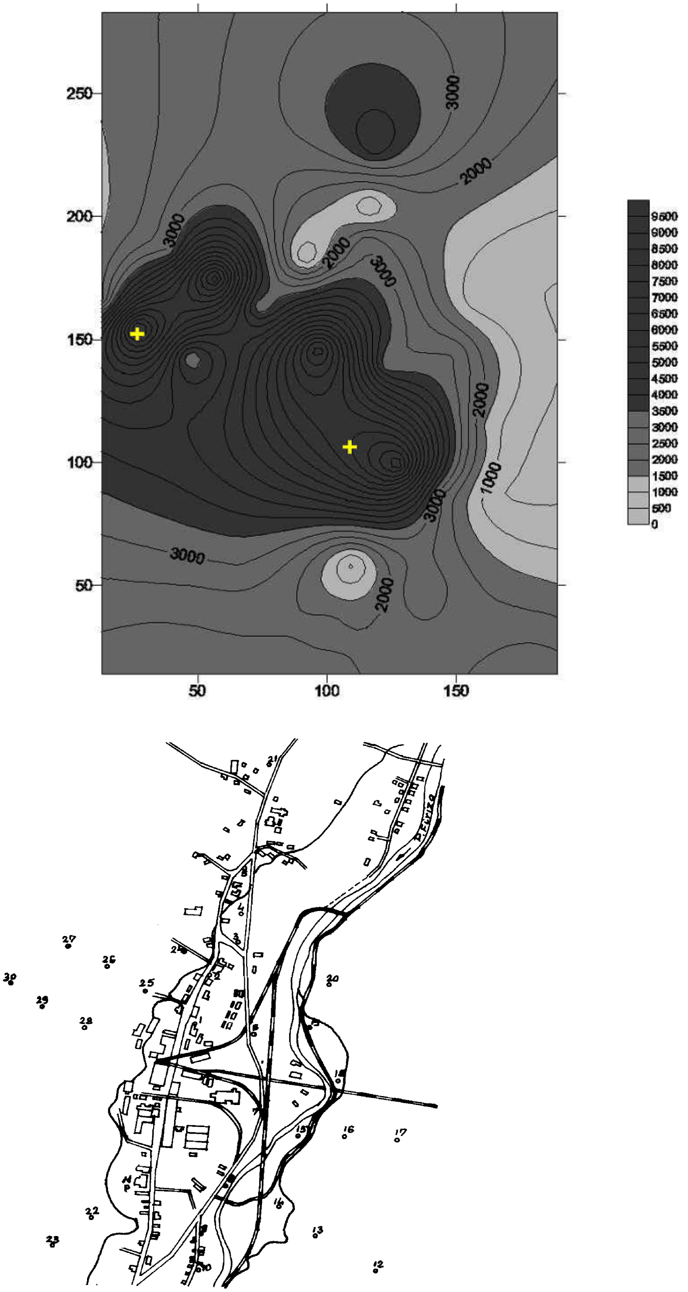


Figure 6. The lead content in top soil of Ferneziu area (lead concentration isolines in ppm) and the Ferneziu quartal map (from Fülöp and Văscan, 2004).

3. Effects of Lead on the People Health. The Lead Level of the Blood in Children in Baia Mare.

Concerning the effects of the pollution with lead on the public health a lot of reports conclude that a prolonged exposition of peoples has a negative influence on the somatic development, decrease the visual acuity and the auditiv treshold. For the childrens the lead reduse the fizical growth and the psyhical development. The IQ of children is diminished and symptomes of irritability and fatigability could be observed. The IQ is diminished also at the childrens borne from mothers which have been exposed at lead contamination.

There is no data for the Baia Mare area of to show a link between the children's blood lead levels and their health problems. However we know that children are more vulnerable to lead poisoning than adults (Cincinnati Children's Hospital Medical Center, 2008). Children activities make it easier for them to be exposed to lead than for adults. Children can be exposed to lead in many ways. Also, compared with adults, a bigger proportion of the lead amount swallowed will enter the blood in children. Children are more sensitive to the health effects of lead than adults. The lead exposure of children is achieved in different ways (Johns Hopkins University Bloomberg School of Public Health, 2007):

- *Prenatal exposure*: women exposed to lead have higher rates of infertility, miscarriage, stillbirth, and premature birth, and are likelier to give birth to babies with minor defects, low birth-weight, and impaired mental development.
- *Acute exposure*: metallic taste in the mouth, muscle weakness, gastrointestinal symptoms (colic, nausea, diarrhea), neurological symptoms (convulsions, coma), brain damage and encephalopathy that could cause death.
- *Chronic exposure*: lead can affect physical growth and can cause anemia, kidney damage, headaches, hearing problems, speaking problems, fatigue or irritable mood, deficits in children's mental development and behavioral problems. Mental disorders in children and adolescents probably linked to lead exposure are: mental impairment, autistic behavior, hyperactivity, attention deficit, aggressive behavior, anxiety disorders, depressive disorder, and psychotic disorder.

Mental Impairment implies an IQ <70, concurrent deficits in adaptive functioning in the following areas: communication, self – help, social skills, use of community resorts, self – direction, academic functioning skills, work, leisure time, health, safety (DSM IV). Most studies have found a correlation between children's mental abilities and lead in their blood. According to these studies, there is a decline in IQ for children exposed to lead (Duke University, 2009; http://www.fi.edu/learn/brain/metals.html#top). Some say that even low-level long-term exposure to lead could cause deficits in cognitive performance and behavior in childhood through adolescence (Bruce et al., 2005; Koller et al., 2004).

Children exposed to lead seem to have very low levels of concentration, are very disruptive, and have violent tendencies (Nigg et al., 2008, 2010). These symptoms can be found in diagnostics such as Attention-Deficit/Hyperactivity Disorder (ADHD) and Conduct Disorder. **ADHD** describes symptoms of inattention, hyperactivity and impulsivity. Often can be related with Conduct Disorder, Oppositional Defiant Disorder or Learning and Communication Disorder. The disturbance in behavior causes significant impairment in social or academic functioning. **Conduct Disorder** implies behaviors in which the basic rights of others or major age-appropriate societal norms or rules are violated (DSM IV).

The ability of lead poisoning to induce symptoms of autism could be relevant to cases of preexisting pervasive developmental disorders irrespective of etiology. Such individuals have a greater propensity to engage in pica and, as a result, are more likely to become lead poisoned. In such cases, lead poisoning can be expected not only to negatively impact the neurocognitive functioning, but also to potentially exacerbate the preexisting symptoms of autism. **Autism** is a developmental disorder with impairment in social interaction, qualitative impairments in communication, repetitive and stereotyped behaviors, interests, and activities, delays in symbolic or imaginative play (DSM IV).

Low-level lead exposure may disrupt brain processes, such as those involving the neurotransmitters catecholamine and serotonin that are associated with depression and panic disorders, more likely to develop in teenagers or young adults. **Panic Disorder** is defined as a period of intense fear or discomfort, in which the following symptoms developed abruptly and reached a peak within 10 min: palpitations, sweating, trembling or shaking, sensations of shortness of breath, feeling of choking, chest pain, nausea or abdominal distress, feeling dizzy, unsteady, lightheaded, or faint, de-realization or depersonalization, fear of losing control or going crazy, fear of dying, paresthesias, chills or hot flushes. These attacks are followed by persistent concern about having additional attacks and worry about the implications of the attack or its consequences. **Depressive Disorder** needs the development of new symptoms such as: depressed mood, loss of all interest and pleasure, irritable mood most of the day, appetite or weight disturbance, sleep disturbance, fatigue or loss of energy, poor concentration or indecisiveness, for at least 2 weeks (DSM IV).

Some authors have suggested that studies are required to further establish, and to investigate possible biological mechanisms involved in the relationship between elevated blood lead levels during the second trimester of pregnancy and the development of schizophrenia (Naylor, 2005). **Characteristic symptoms for schizophrenia** are delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior or negative symptoms (affective flattening, alogia, or avolition) (DSM IV).

TABLE 3. Lead toxicity: standards and regulations for lead (USA).

Agency	Media	Level	Comments
CDC	Blood	10 μg/dL	Advisory; level for individual management
OSHA	Blood	40 μg/dL	Regulation; cause for written notification and mediacal exam
		60 μg/dL	Regulation; cause for medical removal from exposure
ACGIH	Blood	30 μg/dL	Advisory; indicates exposure at the treshold limit value (TLV)
OSHA	Air (workplace)	100 μg/m^3 100 μg/m^3	Regulation; PEL (8 h average) (general industry) Action level
CDC/NIOSH	Air (workplace)	100 μg/m^3	REL (non-enforceable)
ACGIH	Air (workplace)	150 μg/m^3	TLV/TWA guideline for lead arsenate
		50 μg/m^3	TLV/TWA guideline for other forms of lead
EPA	Air (ambient)	0.15 μg/m^3	Regulation; NAAQS; 3-month average
EPA	Soil (residential	400 ppm (play areas) 1,200 ppm (non play areas)	Soil screening guidance level; requirement for federally funded projects only (40 CFR Part 745, 2001)

The pregnant wimin and the very young childrens up to 4 years old are the most vulnerable groups at the effects of lead exposure. The very young child has a specific comportament as he introduce in his mouth the hands and all kind of objects as a way of exploring the environment. This way, within a contaminated environment the child could ingest significant amounts of lead.

Actually the limit of the no effect on the health is accepted 10 μg/dL of lead in the blood (Table 3) as regulated by certain American agencies and by WHO. But this value was established only in 1991, before there was a progressively diminish of the health limit value during the time (Figure 7).

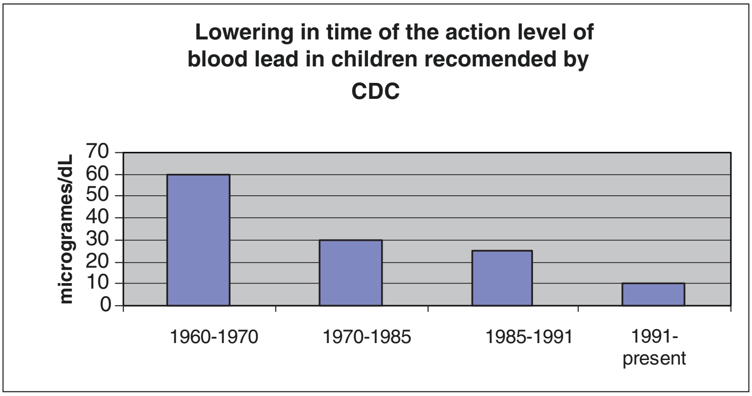


Figure 7. The lowering in time of the action level of the lead level in children's blood.

An invesigation made in 1992 on children aged between 3 and 6 years in different areas of Baia Mare town found high levels of lead in their blood and of arsenium in their urine (Gurzău, 2000). The values of lead level have been differentiated on the territory of the town as follows:

- the group of children living in the western area (the less exposed area) of the town had the lowest values (in average 15.5 µg/100 mL),
- another group of inhabitants in the eastern area exposed mainly to the pollution from a copper smelting factory (Phoenix) had average values (28 µg/100 mL),
- the highest levels have been reported for the third group of children in North-Eastern area (Ferneziu area) nearby ROMPLUMB (32.4 µg/100 mL average).
- The arsenium content in urine had the highest levels for the group from the eastern area (6.44 µg/L average).

Another investigation made by ECOCENTER NGO in 1998/1999 reported the following results (Bindea and Lobontiu, 1999):

- For a mixed group of children from all the area of the Baia Mare town the lead levels in blood were (in 1998):
  - <10 µg/100 mL at 12.5% from the tested children;
  - 10–20 µg/100 mL at 37% from the tested children;
  - 20–30 µg/100 mL at 20% from the tested children;

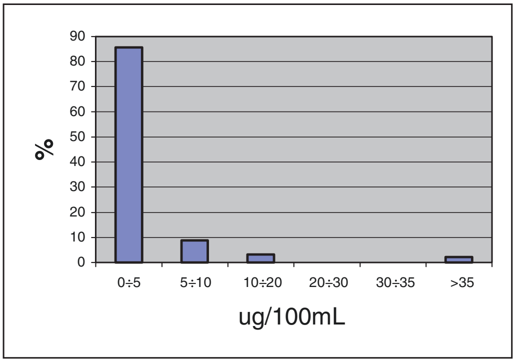
  - 30–40 μg/100 mL at 15.5% from the tested children;
  - 40–50 μg/100 mL at 8% from the tested children;
  - >50 μg/100 mL at 7% from the tested children.
- For a group of children inhabitants in vicinity of the copper smelter Phoenix (in January 1999):
  - >65 μg/100 mL at more than 60% from the tested children;
  - 50–65 μg/100 mL at 15% from the tested children;
  - 40–50 μg/100 mL at 9% from the tested children;
  - <40 μg/100 mL at less than 16% from the tested children;
- For a group of children inhabitants in vicinity of the lead smelter ROMPLUMB (in February 1999):
  - The lead level in blood was between 50 and 65 μg/100 mL at 85% from the tested children.

During the last years, the Public Health Authority of Maramures County is doing an epidemiological study on the lead level in the blood for children and for pregnant women in Baia Mare. It is a biological screening of the population from Baia Mare in the aim to establish the lead exposure and the risk connected with.

A first investigation has been made in 2008 on a group of 100 pregnant women uniformely distributed in all the quartals of Baia Mare town. The lead levels in blood have been mesured using kits for quick testing. The results are presented on Figure 8. It is evident from the reported results that the high majority of pregnant women (94.5%) have a lead level in blood under 10 μg/dL considered as threshold limit. But there are approximately 2% of pregnant women having a value exceeding 35 μg/dL. These cases are from Ferneziu area which indicate a high exposure and an over threshold value that need written notification and medical exam.

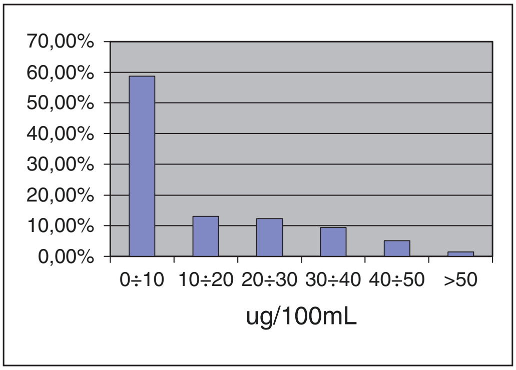
The second screening investigation has been made in 2009 on a group of 138 children aged 0–6 years. The group is representative for the Baia Mare city. The lead levels in blood were mesured using kits for quick testing. The reported results are presented in Figure 9.

From the results it is evident that there exists in Baia Mare about 40% from the very young children having the lead level of the blood over the accepted limit of 10 μg/100 mL. A health risk for these children is possible concerning their mental and physical development. All the registered cases have been notified for educational and health monitoring by the family medcins. Moreover some 6–7% from the children have high lead levels (over 40 μg/100 mL) which indicate a high exposure and health risk.



Intervals of lead levels in µg/dL	Percent from the tested group
0–5	85.7
5–10	8.8
10–20	3.3
20–35	0
>35	2.2

Figure 8. The lead level of the blood of pregnant women in Baia Mare 2008.



Intervals of lead levels in µg/dL	Percent from the tested group
0–10	58.70
10–20	13.04
20–30	12.32
30–40	9.42
40–50	5.07
>50	1.45

Figure 9. The lead level in blood of the children in Baia Mare 2009.

Although the investigation is not finished yet, a comparison with the results reported 10 years ago and 20 years ago could be done. A comparative estimation achieved from the reported results is presented in table no. 4. It is evident from the table that a decreasing of the lead level in children`s blood in Baia Mare is an obvious evolution during the last 20 years. Different factors contributed on this trend and different explanations are available. The decrease of the air pollution with lead in Baia Mare is one of the main factors. The educational programmes and the increase of the educational level concerning the public health and the human rights are also factors that contribute in this tendence.

TABLE 4. Estimation of the evolution in time of the lead level in blood of children from Baia Mare.

Intervals of lead levels in children's blood	1990/1991	1999/2000	2009
0–10 μg/dL	3%	12.5%	58.7%
10–20 μg/dL	4%	37%	13.0%
20–30 μg/dL	6%	20%	12.3%
30–40 μg/dL	20%	15.5%	9.4%
40–50 μg/dL	27%	8%	5.1%
>50 μg/dL	40%	7%	1.5%

Although the general trend in Baia Mare is an important decrease of the lead level in blood, for the Ferneziu area it is not evident that a such evolution exists. The high values reported from the last investigations are located in this area. The high grade of lead contamination in soil and some events of air pollution from the lead smelter are resting strong factors of exposure for the peoples living this area. The education level and the social and economic level of this population is not very high. As a consequence the educational factor in this area will have smaller results.

A special investigation for this special area need to be organized and financed by the authorities within a framework of a large project in the aim to propose a strategy for increase the health and diminish the risks for the population living in Ferneziu area.

4. Conclusions

The most important risk sources for the people of Baia Mare are current air pollution and soil contamination resulted from historical pollution from two large metallurgical factories located within the town. Actually the main sources of heavy metals environmental pollution are the former mining and mineral processing sites and the actual and former metallurgical factories.

Air pollution with lead has been very high during 1980–1990. A decrease in the air pollution with lead from 13.1 to 1.87 μg/m^3 yearly average for 1989 and 1998 respectively, has been registered during the period 1989–1998. Although the accepted level has remained constantly exceeded. Only in 2008 the yearly average for the air pollution with lead has registered a value below the accepted level (0.5 μg/m^3).

The soil pollution with heavy metals (Pb, Cu, Zn, Cd) is generally high in Baia Mare but extremely high levels of lead pollution in soil is registered in Ferneziu area (North West Baia Mare). The population located here are a high level of exposure and high risk of healts.

The investigations on the lead level in blood of the children made during the period of high air pollution reported very high values. A lot of tested children registered dangerous values especially in Ferneziu area. The actual research registered generally values under 10 µg/100 mL but it is evident that there exists in Baia Mare about 40% from the very young children having the lead level of the blood over the accepted limit of 10 µg/100 mL. A health risk for these children is possible concerning their mental and physical development.

A comparative estimation achieved from the reported results indicates a decreasing of the lead level in children`s blood in Baia Mare as an obvious evolution during the last 20 years. Different factors contributed on this trend and different explanations are available. The decrease of the air pollution with lead in Baia Mare is one of the main factors.

A special situation is still continuing for the population of Ferneziu area. High levels of lead in the blood are registered and the decreasing trend is not evident. A special investigation for this special area need to be organized and financed by the authorities within a framework of a large project in the aim to propose a strategy for increase the health and diminish the risks for this population.

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CHANGING CHILDREN'S BLOOD LEAD LEVEL IN HUNGARY 1986–2006

GYULA DURA*, TAMÁS PÁNDICS AND PÉTER RUDNAI
National Institute of Environmental Health
Gyali ut 2-6., 1097 Budapest, Hungary

Abstract. Since 1979 is it known that the lead exposure, which is held harmless for adults, can occur severe behavioral disorders, mental performance and concentration decrease in children and therefore it was necessary to reduce the environmental lead exposure. In Hungary in the mid-1980s the lead content of petrol were progressively reduced. The first specific study on blood lead level of children was carried out in the years 1985 and 1986, and it showed a significant difference between children living in city center and outer districts in Budapest. The significance between blood lead level and lead concentration in air was stronger in children than in adults. In the early 1990s our studies showed, that blood-lead level was still above 10-µg/dL in 8–13% of urban children, higher than 20 µg/dL blood-lead levels were measured only sporadically. Seven years after the introduction of unleaded petrol the average blood-lead level of children was significantly lower, between 1996 and 2006 is the difference more than 60%, however our studies showed also, that petrol lead content is not the only exposure source affecting the blood-lead level. In the next decade is expected moderate elevated blood-lead level in children, which reminds us to reduce the environmental lead pollution.

Keywords: Lead exposure, children, health, blood lead level

1. Introduction. Studies on Lead Exposure in Children

As known, the results of Needleman's (Needleman et al., 1979) examinations three decades ago have attracted attention for lead exposure level, which was held to be harmless in adults, but can lead to heavy behavior disorders, mental and attention deficit in case of children. Worldwide the research groups have

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* To whom correspondence should be addressed: dura.gyula@oki.antsz.hu

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worked on the clarification of the effects of the lead exposure on children by the environment; on grounds of this a claim to reduction of the lead content of petrol has appeared. The results showed much stronger effect in children by the same amount of lead, because the admission and resorption of lead is much higher per body kilogram than in adults. Children are much more sensitive to lead exposure than the adults; many organs of the children are still immature, and thus much more sensitive on toxic effects. The adverse effect of lead exposure is strongly influenced by individual conditions. The gender, age, iron and calcium metabolism and the general immune status play an important role. The nutrition may strongly affect the lead accumulation and clearance (Reddy et al., 2010). The co-exposure of other heavy metals influences the toxic effect of lead as well (Kim et al., 2009). Enzyme polymorphisms, that affect lead toxicokinetics, may be modifiers of effects of lead-exposure (Weaver et al., 2006). Delta-aminolevulinic acid dehydratase (ALAD), which is inhibited by lead, is a most sensitive marker of lead exposure. The three phenotypes of ALAD (1–1, 1–2, 2–2) have a different affinity to lead. The individuals with ALAD 2 allele are more sensitive to lead exposure (Shen et al., 2001), and have higher plasma lead level (Montenegro et al., 2006). The vitamin D receptor (VDR) polymorphisms also affect the plasma and whole blood lead level, individuals with Apal and FokI polymorphysms have lower plasma lead level (Rezende et al., 2008).

2. Studies in Hungary

A lot of studies show the effects of lead exposure on children's health, severe behavioral disorders, mental performance and concentration decrease. Studies in Hungary have also given the same results. The first study in Hungary on lead exposure in cooperation with WHO began in 1985, within the frame of which teeth, hair and blood samples were collected from children with age 7–9 living in city center, medium sized city and village (Winneke et al., 1990). The results show, that the main source of lead exposure was the traffic (Table 1a and b).

TABLE 1a. Sources and Pb levels in air, sedimenting dust and soil of different urban regions of Budapest, Hungary.

Site	Pb source	Pb in air ($\mu g/m^3$)	PM (mg/m^2)	Soil Pb (mg/kg)
Village	industrial	–	14.0	41.4
City centre, Budapest	traffic	5.3	–	298.0
City periphery, Budapest	traffic	2.9	–	100.0
Medium sized town, periphery	–	–	–	12.8
Medium sized town, centre	traffic	2.0	–	35.3
Medium sized town, Heves	traffic	–	–	12.4

TABLE 1b. Lead level in blood, hair and teeth in children living in city, medium sized town and village in 1985.

Site	Pb in blood µg/dL	Pb in hair µg/g	Pb in teeth µg/g
Village	15.6 (±6.8) (n=56)	6.6 (±5.9) (n=56)	1.8 (±1.1) (n=16)
City centre, Budapest	27.3 (±3.2) (n=33)	11.3 (±9.4) (n=34)	2.8 (±1.8) (n=11)
City periphery, Budapest	22.5 (±9.5) (n=37)	4.9 (±3.0) (n=35)	
Medium sized town, periphery	16.6 (±8.4) (n=53)	3.1 (±3.0) (n=66)	
Medium sized town, centre	23.7 (±12.1) (n=45)	3.2 (±2.8) (n=46)	3.4 (±1.7) (n=43)
Medium sized town, Heves	21.0 (±10.1) (n=43)	3.1 (±3.0) (n=42)	

In 1986, a new study was carried out in Budapest to compare the lead exposure in city centre and on periphery (Rudnai et al., 1990). The results showed a moderate difference in lead blood level in adults living in city centre and periphery, but lead blood level of children in the city centre was significantly elevated compared to children living in periphery. Based on collected data 1 µg /m^3 change in air lead concentration results in a 1.2 µg/dl blood lead level increase in adults and 4.2 µg/dl in children. These results explain the heavy decrease of lead blood level in children after the reduction of lead content of petrol (Table 2).

TABLE 2. Blood lead level in individuals in Budapest in 1986.

Study group	Study field	N	PbB (µg/dL)		PbB>12 µg/dL	PbB>20 µg/dL
			average	median	(%)	(%)
Male	Centre	63	14.1	13.0	58.7	14.3
	Periphery	74	13.3	11.0	44.6	10.8
Female	Centre	70	11.4	10.6	38.6	4.3
	Periphery	76	9.3	9.0	17.1	5.3
Pregnant	Centre	17	8.6	9.2	17.6	0.0
	Periphery	38	10.0	9.2	31.6	0.0
Children 7–9	Centre	70	24.8	23.0	88.6	57.1
Years	Periphery	59	7.6	7.2	6.8	1.7

After the reduction of lead content of petrol in 1990s several studies were carried out on blood lead level of children in Hungary. Results of a study in Hungarian cities showed, that the blood lead level of 8–13% of children living in city centers was still over 10 µg/dl, which proves, that there must be another important lead source.

In 1996 studies on other age groups were done. Blood lead level of children in kindergarten was measured, the average blood lead level (6.5 µg/dl) was relatively low (Rudnai et al., 1998). Beside the air lead pollution some other factors were found, which may affect the blood lead level as well. Fruits from indoor store and fruit juice act protecting, whereby drinking coke and second hand smoke are adverse (Table 3).

TABLE 3. Factors affecting blood lead level in children.

Factors	Regression coefficient	P
Age	− 0.0117	0.925
Gender	− 0.4475	0.093
Education (mother)	−0.1716	0.001
Education (father)	−0.1423	0.003
No. of smoker	0.3610	0.033
Traffic	0.1912	0.535
No. of rooms	−0.4615	0.004
Buying fruit in indoor store	−0.6704	0.044
Coke	1.5757	0.000
Fruit juice	−0.5875	0.056

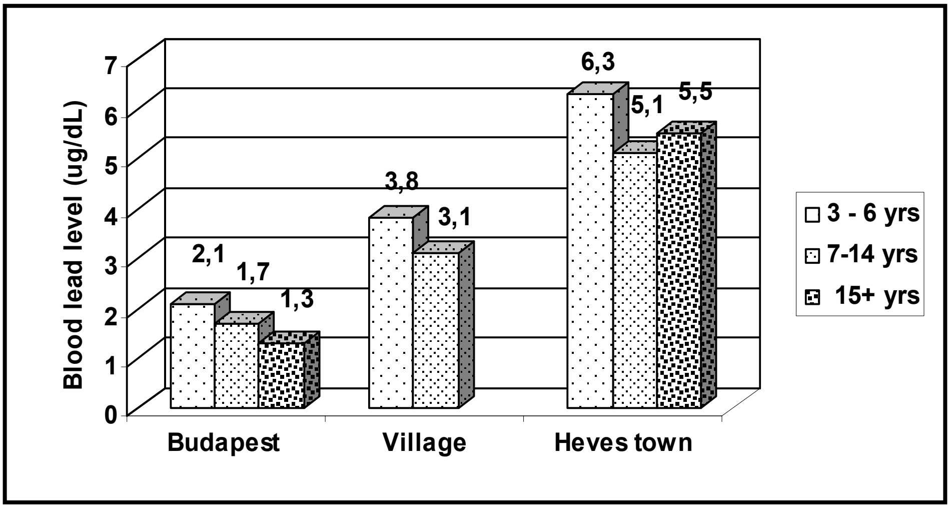

Figure 1. Blood lead levels in different age groups.

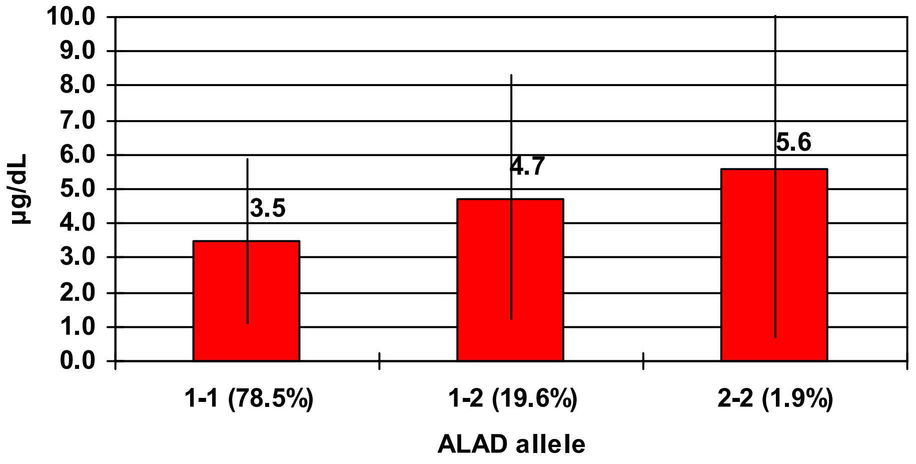

Figure 2. ALAD alleles and average blood lead level.

Ten years after the reduction of lead content of petrol a new study was carried out in Hungary to explore other sources of lead exposure (Rudnai, 2007). The study focused on children in kindergarten living in sites polluted earlier by lead. The average blood lead level was relatively low, but the results showed a higher blood lead level in younger children (Figure 1). Psychosomatic disorders were described in group of children, which had a higher lead level than 15 µg/l.

Studies on polymorphism of enzymes affecting blood lead level were also carried out. The results showed a significant correlation between ALAD polymorphism and blood lead level. In the studies was reported a significant higher proportion of 1–2 ALAD heterozygote in the study group with over 20 µg/l blood lead level than in lower blood lead range (Figure 2).

3. Conclusions

After the reduction of lead content of petrol the lead level of children has become lower, but it is known, that there is no such lead level, which has no affect on children's health. A lot of other lead sources are known: indoor chemicals, indoor smoking/second hand smoke, heavy traffic, water pipe containing lead. One factor, the petrol lead content was eliminated, but there is still a lot of work to discover other lead sources in order to reduce them.

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ANALYTICAL APPROACHES FOR SAMPLING AND SAMPLE PREPARATION FOR HEAVY METALS ANALYSIS IN BIOLOGICAL MATERIALS

ELISABETA CHIRILA[1]* AND CAMELIA DRAGHICI[2]
[1] *Ovidius University of Constanta, 124 Mamaia Bldv,*
900527 Constanta, Romania
[2] *Transilvania University of Brasov, 29 Eroilor Bldv,*
500036 Brasov, Romania

Abstract. Heavy metals concentrations in biological materials are necessary to be known when investigations in environmental or health risk assessment are done. The paper aims to review the modern aspects of the first step of the analytical process, sampling and sample preparation which involve some of the following operations: sample collection (sampling), washing, storage, preservation, followed by sample transformation to the most appropriate form for measurement. New separation techniques, applied for specific analytes extraction or preconcentration from biological matrices and metal speciation are also briefly described.

Keywords: Heavy metals, sampling, biological sample preparation, speciation

1. Introduction

Heavy metals occurrence in biological materials is due to natural or artificial processes and information about their concentration is important for the environmental or health risk assessment. Of the 92 naturally occurring elements, approximately 30 metals and metalloids are potentially toxic to humans: Ag, Al, As, Au, B, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Hg, Li, Mn, Mo, Ni, Pb, Pd, Pt, Sb, Se, Sn, Sr, Te, Ti, V and W (Ortega, 2002). Some of the above mentioned metals (elements) have essential role in living organisms and only when their concentration is higher than a particular limit, may produce diseases.

Biological material considered for metal analysis could be (i) biota samples (living organisms for environmental and toxicological studies) and (ii) human

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* To whom correspondence should be addressed: echirila@yahoo.com

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biological fluids (urine, blood, serum, saliva etc.) or tissues (hair, bones, nails etc) (Fatima Reis et al., 2007a, b; Lalor et al., 2007; Zheng et al., 2008; Kangmin et al. 2009; Schulz et al., 2009; Son et al., 2009). For biological monitoring of heavy metal exposure in occupational toxicology, usually whole blood and urine samples are the most widely used and accepted matrixes to assess internal xenobiotic exposure (Bader et al., 1999; Wiwanitkit, 2008).

The chemical measurement process is defined as the set of operations that comes between uncollected, unmeasured, untreated samples and the results it provides. The main steps of the analytical process and their importance in the quality of results are presented in Figure 1 (Draghici et al., 2010).

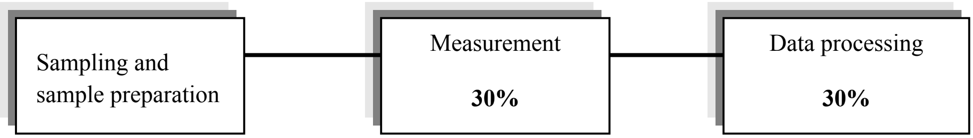

Figure 1. The main steps of the analytical process.

The aim of this study is to review the modern aspects of sampling and sample preparation, the first step in the process of measuring the concentration of metals in biological materials.

Also called "preliminary operations" the first step of the analytical process involves some of the following operations: sample collection (sampling), washing, storage, preservation, followed by sample transformation to the most appropriate form for measurement.

2. Sample Collection

When the aim of the experimental work based on biomonitors is environmental studies, the organisms used as biomonitors must: (i) be easy to identify; (ii) have adequate dimensions; (iii) be long living; (iv) be abundant; (v) and be less mobile etc. These samples are washed with deionized water before storage, stored in inert material recipients and preserved through freezing (Chirila et al., 2006).

In occupational toxicology or clinical studies for trace metal testing, blood or urine samples are commonly required (Heitland and Koster, 2006). When performing venipuncture, the nurse or phlebotomist collecting the sample should observe universal precautions for prevention of transmission of blood borne pathogens. Trace metal contamination is a potentially serious problem induced to samples for trace metal analysis. Metals are present in the materials used to manufacture rubber stoppers and lubricants used in blood collection tubes. Therefore, special tubes with lubricant-free stoppers are required. Samples for lead analysis require whole blood because the lead is primarily within the red blood cells. Special tubes containing heparin or EDTA (ethylenediaminetetraacetic acid) are used for this purpose. These have a tan colored stopper and are certified to be lead free. Other trace metals are usually measured in serum or urine. If serum is used, the blood must be collected in a tube having a navy blue stopper. The only exception is sampling for iron determination which is present in sufficient concentration in serum or plasma to allow the use of regular blood collection tubes. In addition, when performing analysis of any trace metal, the water used must by deionized, and the reagents must meet or exceed purity standards (Goulle et al., 2005).

Human hair has proved to be a vehicle of excretion of substances from the human body, heavy metals among them, and their concentrations in hair are up to 10-fold higher than the levels found in blood or urine samples, thus reducing contamination during analysis to a minor problem. In contrast to blood and urine specimens, hair must be cleared of external contaminants prior to element analysis. Moreover, the determinations in hair can hardly be interpreted as representing an exposure test in the classic sense, but may substantially help in monitoring excessive exposure. In other words, hair samples can help to monitor environmental or occupational exposure and appear to be suitable for use in pilot prospective studies as well as for identifying the population groups at specific risk and excessively contaminated areas. On the other hand, while blood and urine concentration clearly reflect recent exposure, hair reflects past exposure providing an average of their growth period (Bader et al., 1999).

At present it is also appropriate to measure levels of heavy metals in saliva in order to determine whether or not these compounds in salivary samples can provide a reliable index of occupational exposure (Wang et al., 2008).

As an example, axilary hair was washed by ultrasonic cleaning (15 min) in a 0.5% non-ionic detergent solution (Triton X-100), the detergent was removed by rinsing with Milli-Q water, washed by ultrasonication for 15 min in a 95% ethanol solution, and again washed with Milli-Q water. The cleaned hair was dried at 70°C overnight. Axilary hair was ground to a fine powder using a manual agate mortar (Olmedo et al., 2010).

Other authors suggest hair and nail as biopsy materials as more attractive biomarkers in assessing heavy metals environmental exposure. All the fingernails of the subjects were cleaned by trained personnel using surgical spirit, followed by non-ionic detergent. By use of metallic free cotton wool wetted with acetone, they were cleaned, rinsed with water, dried with a clean towel. All the fingernails were clipped from the same subject every 2 weeks and satisfactory samples were obtained after a period of 7 months. They were kept in a labeled plastic container securely closed, until analyses (Hussein Were et al., 2008).

3. Sample Preparation

Some of the major functions of sample preparation are:

a. to degrade and solubilize the matrix;
b. to release all metals for analysis;
c. to extract metals from the sample matrix into a solvent more suitable for the used analytical method;
d. to bring metals present at very low levels them into a concentration range suitable for analysis;
e. to separate a single analyte or group of analytes from other species that might interfere in the analysis;
f. to dilute the matrix sufficiently so that the effect of the matrix on the analysis will be constant and measurable;
g. to separate different chemical forms of the analyte for individual determination of the species present.

The major concerns in the selection of sample preparation methods for metal analysis are (i) the type of matrix in which the analyte exists; (ii) the type of the analyte; (iii) the concentration range of the analyte; (iv) the analytical method to be used.

Most of the quantitative techniques for traces analysis are using liquid samples, except for X-ray fluorescence, which often is used on solid samples. Therefore, the complex solid samples (which can be particulate matter from air, soil, or biota) or the organic liquids are subject to a disaggregation process (also called digestion or mineralization). The extraction of the species of interest from the solid samples can be the most difficult aspect of the analysis. Adequate techniques are necessary to convert the solid matrix into solution.

Wet digestion in acid solution, dry ashing, and extraction of the analyte from the sample without total matrix destruction are common sample preparation methods. Sample digestion can be done as follows: (i) wet digestion with strong acids like HCl, HF (if silicates are present), HNO_3; (ii) wet digestion with mixtures of strong acids with oxidizing agents like royal water, $HClO_4$ or H_2SO_4 with H_2O_2; (iii) melting with fondants such as NaOH or $K_2S_2O_8$ followed by solubilization in water; (iv) calcinations followed by solving the ash in an acid (dry-ashing); (v) microwave digestion, a modern technique combining pressure and temperature which has the advantage of substantial time-saving and less reagents use.

Dry ashing is useful for moist samples, such as food or botanical samples, because it destroys large amounts of wet organic matter easily and quickly. However, if the metal analyte is present in a volatile form (methyl mercury) dry ashing can cause loss of analyte. Many sample matrices, both organic and inorganic, can be dissolved by heating in a strong oxidizing acid solution.

Other samples can be treated by extracting the metals from the matrix. This method is frequently used for water samples, where a chelating agent may be used to complex the metals of interest, enabling their easy separation from the aqueous matrix.

Sample lyophilization (freeze-drying and high-vacuum procedure) is a highly effective alternative for biological and food samples. Water is removed and the matrix structure broken, which facilitates dissolution of the resulting residue in an appropriate solvent (e.g. an acid) under mild conditions.

As an example, samples were frozen in sealed plastic bags for transportation from a hospital to the laboratory for further analysis. Prior to analysis, blood samples were lyophilized for 72 h at −60°C and low pressure (ca. 0.1 atm). The lyophilization time was optimized for total removal of water. Following this procedure, a powder was obtained of each sample in a polyester mill, and the samples were stored under controlled humidity conditions. For the analysis the samples were pressed into pellets of 2.0 cm in diameter and 1 mm thickness. A minimum of three pellets of each sample were produced to reduce the error of analysis. Each pellet, without any substrate, was glued on a Mylar film and put directly on a sample holder in the X-ray beam for element determination (Custodio et al., 2005).

Since many of these analyses are done to determine traces of metals, and because the reagents used for matrix destruction are often quite aggressive, prevention of sample contamination from the containers or from the reagents themselves requires constant care. In addition to samples contamination, the loss of analyte during the preparation of the samples is also possible. Metal ions may adsorb on surfaces, especially on glass surfaces. Silica vessels are less likely to adsorb metals. Some metals (iron, mercury, gold, and palladium), may be removed from samples when they are heated in platinum crucibles. These adsorbed ions are not only lost for the analysis, but may also remain on the container walls to contaminate future samples. Volatilization is another major cause of analyte loss and can be serious for the more volatile metals when samples are dry-ashed. Cadmium, lead, mercury, selenium, and zinc are especially volatile at temperatures below 500°C. Volatility depends rather strongly on matrix and on the form of the present metal. Metals that have been found to be well recovered at a certain temperature in one type of sample may be subject to losses by volatilization in a different matrix or sample.

4. Analytical Separation Techniques

It is not always necessary or required to digest the entire sample in order to free the metals for analysis. Therefore some specific methods were developed in order to separate (isolate) the analyte of interest from the rest of the matrix. From all these separation techniques, two of them will be further presented.

For the extraction with supercritical carbon dioxide, metals are firstly chelated with an adequate ligand, such as a derivative of dithiocarbamate. It has been found that while the solubility of metals chelates with sodium diethyl dithiocarbamate in carbon dioxide is quite low, the solubility can be significantly increased by substituting a longer chain alkyl group for the ethyl groups on the dithiocarbamate. Even better extractions were obtained when the ethyl groups of the diethyl dithiocarbamate were fluorinated. The solid sample is placed in the preheated extraction thimble, and the modified CO_2 is added to the desired extraction pressure. The system is held at constant extraction temperature and pressure. At the end of the process, the system is vented into a collection vial containing chloroform. This is followed by a dynamic flush of the system with the CO_2 solvent at the same temperature and pressure. For example, mercury complexed with fluorinated diethyl dithiocarbamate was extracted from dried aquatic plant material with 95% efficiency by methanol-modified CO_2 using supercritical fluid extraction technique (Wang and Wai, 1996).

After the destruction of organic matter, biological samples are transformed in solutions which contain mixtures of metal ions in concentrations varying from too small to be measured up to those normal. When the sample contains concentrations of analyte below the detection range of the analytical instrument used for the determination, a concentration step is often required. Metals can be concentrated from solution by solid-phase extraction (SPE). SPE usually involves passing the solution through a column, cartridge, or disk containing a solid material (adsorbent) that more or less specifically binds the metal ions present in solution. The involved mechanism in solid phase extraction process is presented in Figure 2 (Draghici et al., 2010).

The solid-phase extractant may be an ion-exchange resin, a chelating resin, or other material designed to bind all cations or anions, or to bind specific groups of ions. In addition, this method may be used in conjunction with a dissolved chelating agent, using an organic-binding solid-phase extractant to separate the chelated metal from the solution.

Recent development of self-assembled monolayers on mesoporous ceramic supports have led to very efficient, rapid, and highly selective materials for sequestration of metals. The ceramic support material incorporating copper ferrocyanide ethylene diamine was found to remove 99% of the cesium from a 2 ppm solution within 1 minute.

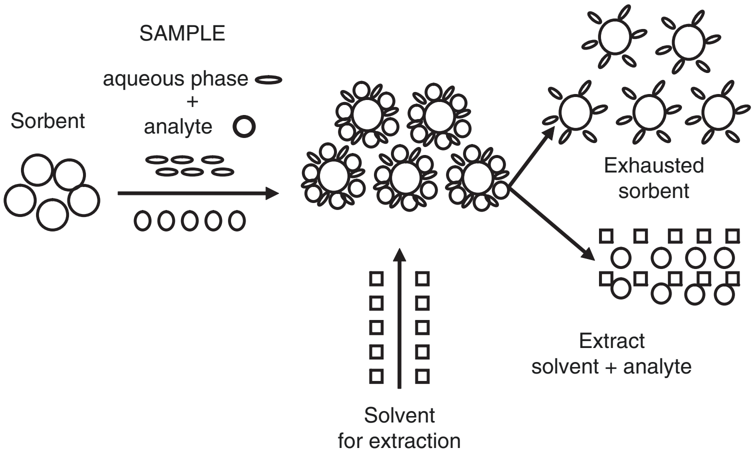

Figure 2. Solid phase extraction process.

The sorption was not hampered by acid or high concentrations of sodium or potassium. The adsorbed metal can be removed by eluting with an oxidizing agent. The sorbent can then be regenerated by using a reducing eluent. A similar material treated with alkyl thiol can be used to sequester mercury, lead, and silver, with high efficiency. The metals can be recovered using an acid eluent (Lin et al., 2001).

5. Hydride Generation Methods

Some metals, for example arsenic and selenium, are difficult to analyze by atomic absorption spectrometry (AAS) because their analytical wavelengths are subject to considerable interference.

These metals however, are readily converted to gaseous hydrides by treatment with strong reducing reagents such as sodium borohydride ($NaBH_4$) and are separated from the sample matrix; as a result, interferences are strongly reduced. The hydrides formed in the reaction chamber are purged into a heated cell of the atomic absorption spectrometer (AAS) and are decomposed to free atoms for measurement.

The kinetics of the borohydride reduction of the various arsenic and selenium species differ and must be taken into account. The different oxidation states give different analytical sensitivities, and different interferences are found for each compound. The optimal pH for reduction to hydride of selenium and arsenic in different oxidation states is also different. Therefore, unless speciation is to be done, it is best to bring all the analyte to a common oxidation state before reaction with borohydride.

For example, arsenic acid, containing As(V), is reduced considerably slower than As(III) compounds. The As(III) is instantaneously reduced, giving a rapid injection of hydride into the instrument. Therefore, it is best to ensure that all the arsenic is in the As(III) state before adding the borohydride. This is accomplished by digesting the original sample with acid, yielding As(V). This is quantitatively reduced to As(III) with sodium or potassium iodide. For example, KI would be added to convert As(V) to As(III). Then a 3% $NaBH_4$ solution in 0.5% NaOH is added and the hydride flushed into the instrument (eg. AAS) for analysis. The sample is then ready for reaction with the sodium borohydride (Carrizales et.al, 2006).

Se(VI) is not readily reduced by sodium borohydride, and samples containing it must be pre-reduced. Samples containing organic selenium compounds or complexes may require digestion with an oxidizing agent, either alkaline hydrogen peroxide or potassium permanganate. The excess of permanganate is removed by reaction with hydroxylamine, and any chlorine formed is removed by boiling in an open container. These digestions leave the selenium in the Se(VI) oxidation state. It is then necessary to reduce this to Se(IV) by boiling with HCl. Se(VI) is rapidly reduced, giving a sharp injection of hydride into the instrument. Biological samples should be dry-ashed before analysis. Magnesium oxide is used as an ashing aid. The ashed sample is taken up in HCl solution, and the oxidation state of the analyte is adjusted (Kebbekus, 2003).

6. Metal Speciation

Knowledge of the forms that an element can have is of primary importance because their toxicity, mobility, bioavailability, and bioaccumulation depend on the chemical species.

The interaction of metals with biological organisms is highly dependent on their chemical form. Some metallic species may be able to chemically bind directly with proteins and enzymes, others may adsorb on cell walls, and still others may diffuse through cell membranes and exert a toxic effect. Toxicity is organism dependent and occurs when an organism is unable to cope either by direct use, storage, or excretion with additional metal concentration.

The impact of some metals is strongly related to their chemical form rather than to their total concentration. For instance, arsenic is generally toxic in both its As(III) arsenite and As(V) arsenate forms, but is nontoxic in its organic forms, such as arsenocholine. Mercury, on the other hand, is toxic in all forms but is substantially more toxic as methyl mercury than it is in the elemental state. Chromium in the Cr(III) oxidation state is less toxic and less soluble than it is in the Cr(VI) state.

Therefore, the total metal concentration is inadequate to fully describe a sample and speciation of the present metals is sometimes required. Speciation can be defined functionally, operationally or chemically. A functional definition specifies the type of role that the element may play in the system, from which the sample was taken, is probably closest to what the end user of the information really wants to know but is the most difficult for the analytical chemist to determine. For instance, a functional definition might be "that copper which can be taken up by plants" or "iron that can be absorbed from a pharmaceutical". The operational definition is considerably more practical. Operationally determined species are defined by the methods used to separate them from other forms of the same element that may be present. "Metals extracted from soil with an acetate buffer" or "Lead present in airborne particles", "Soluble/insoluble metal species" etc. are examples of operational speciation. When operational speciation is done, careful documentation of the protocol is required, since small changes in procedure can lead to substantial changes in the results. Standardized methods are recommended, as results cannot be compared from one laboratory to another unless a standard protocol is followed (Quevauviller, 1998).

Chemical speciation means the determination of particular chemical species, as when arsenic content is separated into As(III), As(V), monomethyl arsenic acid, and dimethyl arsinic acid using ion-exchange chromatography.

Chemical speciation is a developing field and presents difficulties to the analyst. The concentration of present metals may be near the level of detection for the analysis, and when the total concentration is further subdivided into several different species, greater analytical sensitivity is required. If the metals present in a sample are to be separated into their different forms, the initial separations are often carried out during the sample preparation.

Some metals are of particular interest in environmental samples, and specific methods for their chemical speciation have been developed. Arsenic, chromium, and mercury are important in this respect, being known that they exhibit very different toxicities in their different forms.

6.1. ARSENIC SPECIATION

Arsenic exists primarily as As(III) (arsenious acid – H_3AsO_3 and its salts), as As(V) (arsenic acid – H_3AsO_4 and its salts) or organic arsenic compounds such as monomethylarsonic acid and dimethylarsinic acid. The organic compounds are generally found at low levels and are not as toxic as the inorganic species. Therefore, speciation for arsenic tends to be directed toward the determination of As(III) and As(V). The samples in aqueous solution are separated using ion-exchange chromatography (eg. Xie et al., 2006).

6.2. CHROMIUM SPECIATION

Chromium exists in two major species: asCr(III) cation and as Cr(VI) chromate and dichromate anions. Cr(VI) is considerably more dangerous in environmental samples because it is more soluble and also more mobile in the environment, and therefore more toxic.

An example of Cr speciation is based on the following procedure. A 0.05 M ammonia buffer is used at pH 8, with ultrasonication to extract the Cr(VI) from samples; Cr(VI) is than adsorbed from the extract on an anion-exchange column (like Dowex 1-X8). The adsorbed analyte is eluted with 10 mL of 0.5 M ammonia buffer at pH 8. The Cr(III) is determined by difference from the total Cr measurement. A cationic ion-exchange resin can also be used for the Cr(III) ions absorption. Cloud point extraction technique was also applied for the speciation of Cr utilizing ammonium pyrrolidine dithiocarbamate (APDC) as a chelating agent for both oxidation forms of Cr, by properly controlling the reaction conditions for the treated samples (Paleologos et al., 2001).

6.3. MERCURY SPECIATION

Organic mercury species are methyl mercury which is of much greater concern when health effects are considered, as it is much more toxic than ionic or free mercury. Methyl mercury is also much more likely to bioaccumulate, leading to serious contaminations, especially of fish.

The speciation for mercury can be accomplished by derivatizing the methyl mercury and Hg^{2+} with sodium tetraethylborate, $NaBEt_4$. The volatile MeHgEt, from methyl mercury, and $HgEt_2$, from Hg^{2+} species formed are purged from the sample solution and separated in a GC column. An atomic emission spectrometer is used as a detector (Grinberg et al., 2003).

Methyl- and inorganic mercury were extracted from human whole blood samples, as their diethyl dithiocarbamate complexes, into toluene and butylated by using a Grignard reagent. The mercury species were then separated by gas chromatography (on a 12 m non-polar DB-1 capillary column) and detected by a microwave-induced plasma atomic emission spectrometric (GC-MPD) system (Bulska et al., 1992).

6.4. SAMPLE PREPARATION FOR METAL DETERMINATION IN HUMAN BIOLOGICAL MATERIALS

Table 1 presents some examples of specific sample preparation for metal determination in human biological materials using atomic absorption and atomic emission spectrometric techniques.

TABLE 1. Sample preparation for heavy metals determination in human tissues or fluids using atomic absorption and emission spectrometry.

Sample (metals, technique)	Device	Preparation	References
Blood urine (Li, Be, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Ba,W, Pt, Hg, Tl, Pb, Bi, U, ICP-MS)	Heparinized vacutainer tubes non-preservative polystyrene vials	0.4 mL whole blood + 3.5 mL HNO_3 (Triton 0,1%), + centrifugation 0.3 mL plasma + 2.7 mL HNO_3 0.4 mL urine 1 + 3.6 mL HNO_3 0.6 mL urine 2 + 2.3 mL HNO_3	Goulle et al., 2005
Blood (frozen at −18°C) urine (frozen at −18°C) (Cd, Hg, Pb ETAAS)	S-Monovette Sarsted tubes containing heparin and appropriate siliconized needles	5 mL HNO_3 conc. + 1 mL H_2O_2 30% digestion in microwave oven; evaporation to 0.1 mL dilution to 5 mL with demineralized water	Batariova et al., 2006
Blood (venous puncture) urine (As, Pb, GFAAS)	Lead-free tubes containing EDTA as anticoagulant; stored in plastic bottles; kept frozen until analysis	Sample + matrix modifier 0.5–3.0 mL wet digested with nitric, sulfuric, and perchloric acids	Carrizales et al., 2006
Blood (Pb, ETAAS)	Tubes containing EDTA	Stored at 4°C until analysis	Mathee et al., 2006
Blood (Pb, GFAAS)	Free disposable syringe and needle	Transferred to a lead-free tube, kept in an ice-box during transportation and stored at 4°C	Youravong et al., 2006
Blood (Pb, AAS)	Li-heparin sample tubes (Sarstedt, Monovette) with EDTA as anticoagulant	200 µL blood + 1.2 mL mixed matrix modifier solution (0.2% HNO_3, 0.5% $NH_4H_2PO_4$); left 5 min.; centrifuged at 3,000 rpm)	Olivero-Verbel et al., 2007; Borosova et al., 2008
Nails (Pb, Cd, Ca, Zn, Fe, AAS)	Clipped every 2 weeks (7 months), kept in labeled plastic container	Cleaned, kept overnight at room temperature with 10 mL of 6:1 mixture of concentrated HNO_3 and $HClO_4$, heated at 180°C about 1 h	Hussein Were et al., 2008

(*continued*)

(*continued TABLE 1.*)

Sample (metals, technique)	Device	Preparation	References
Saliva whole blood urine axilary hair (Cr, Cd, Mn, Ni, Pb, GFAAS)	Polypropylene tubes acid pre-cleaned polyethylene containers	Washed hair; 30 min. digestion in microwave owen with 1 mL HNO_3, 0.5 mL HCl, 2 mL H_2O_2 and 2 mL H_2O	Olmedo et al., 2010
Red blood cells cord blood maternal blood (Hg, Pb, As, Cd, Se, CVAAS, ICP-MS)	Tubes of with sodium heparin	Centrifuged 10 min. at 3,000 rpm and stored at -80°C; 100 mg RBC + 100 µL internal standard, diluted with matrix solution 0.05 mL concentrated ammonia; 1 mL 0.01 M EDTA + 0.7 mL Triton X-100 + 20 mL butanol/L	Sakamoto et al., 2010
Cord blood (stored at -200°C) (Cd, Pb, HR-ICP-MS)	Polypropylene tubes filled with 0.5 mL Na_2EDTA anticoagulant	200 µL cord blood, 1 mL milliQ water, 1 mL distilled nitric acid and subsequently 1 mL hydrogen peroxide suprapure, mixed in closed vessels and subjected to increased pressure and temperature.	Koppen et al., 2009
Cord blood (K, Ca, Fe, Pb, Cu Zn,Br, Rb, EDXRF)	Sealed plastic bags, frozen	Lyophilisation 72 h at -60°C and 0.1 atm	Custodio et al., 2005

Abbreviations: AAS – atomic absorption spectrometry; CVAAS – could vapor AAS; EDXRF – energy dispertion X-ray fluorescence; ETAAS – electrothermic AAS; GFAAS – grafite furnance AAS; ICP-MS – inductively coupled plasma – mass spectrometry; RBC – red blood cells.

7. Conclusions

Sampling and sample preparation procedures applied for heavy metals analysis in biological materials, especially when the concentrations are as trace levels need to be chosen carefully, taking into account of all probable contamination processes which could occur.

The purity of used reagents, the selection of proper materials for the digestion vessels and of proper digestion temperature are important tasks to be considered.

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HEAVY METALS DETERMINATION IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

CAMELIA DRAGHICI[1]*, CRISTINA JELESCU[2], CARMEN DIMA[2], GHEORGHE COMAN[1] AND ELISABETA CHIRILA[3]
[1] *Transilvania University of Brasov*
29 Eroilor Bldv., 500036 Brasov, Romania
[2] *Apa Company, Brasov*
13 V. Tepes Str., 500092 Brasov, Romania
[3] *Ovidius University of Constanta,*
124 Mamaia Bldv. 900527 Constanta, Romania

Abstract. Heavy metals are present as elementary compounds or mineral deposits in nature, from which they are extracted and processed for different purposes. During these processes, heavy metals are released in the environment and reach the atmosphere, waters, soils, and biota (plants and animals). Heavy metals release into the environment is of great concern, due to their ability to concentrate and accumulate, in different stable forms and in different media. Quantitative determination methods and techniques are developed in order to provide valid results about the heavy metals content in different matrixes. This study presents the main analytical techniques available for heavy metals determination in environmental and biological samples, as well as an example of method validation and uncertainty estimation for Cu^{2+} determination in waste-water samples.

Keywords: Heavy metals, analytical methods, analytical techniques, method validation, uncertainty estimation

1. Introduction

"Heavy metals" is a very imprecise term, used loosely to refer to both the element and its compounds. It is based on categorization of different criteria (Duffus, 2002):

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* To whom correspondence should be addressed: c.draghici@unitbv.ro

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- atomic number or atomic weight (relative atomic mass);
- density, which is rarely a biologically significant property;
- other chemical properties or toxicity.

Most commonly, heavy metals is a generic term used for metallic elements and metalloids (semimetals) and their compounds having the atomic weight higher than 40.04 (higher than the atomic weight of calcium). In combinations, depending on their electronic structure, some of the heavy metals present one or multiple valences, because of their ability to bind with other atoms.

Due to the fact that most of the measurements for their determination are carried out in aqueous solutions, the stable soluble forms of the heavy metals should be known:

- cations – heavy metals with single valence (Ni^{2+}, Zn^{2+}, Cd^{2+}), heavy metals with multiple valences, in their lower valence form (Mn^{2+}, Fe^{2+}, Pb^{2+}, Sn^{2+}, Cr^{3+},) and some in their higher valence form (Cu^{2+}, Fe^{3+});
- oxoanions – heavy metals with multiple valences (AsO_3^{3-}, AsO_4^{3-});
- soluble complexes – heavy metals binding by coordination different inorganic or organic ligands;
- (un)ionized organometallic compounds.

The aim of this study is to give an overview of the main analytical methods and techniques used for heavy metals determination in environmental and biological samples (aqueous ones) as well as to present a validation procedure, and uncertainty assessment for Cu^{2+} determination in standard solution and wastewaters.

2. Analytical Methods and Techniques for Heavy Metals Determination

Analytical measurements, as an integral part of environmental management, can provide valuable information for:

- routine monitoring of regulated contaminants, that ensures compliance with allowable levels and indicates any hazardous situation;
- the location and extent of contamination for contaminated sites remediation;
- health effects assessment, followed by prioritizing contaminants for regulation.

Analytical techniques are available for different determinations of heavy metals:

- heavy metals quantitative determination, for multielement analysis;

- element-specific methodologies for individual determination of metals and metalloids;
- heavy metals speciation.

The analytical methods and their related techniques are based on a specific property of the analyte that can be measured by an appropriate system (equipment). Table 1 presents the principles of the analytical methods and techniques for heavy metals determination in different samples, detailing their applicability, as well as some advantages and disadvantages (Ortega, 2002).

TABLE 1. Analytical methods and techniques for heavy metals determination in environmental and biological samples.

Method and technique	Symbol	Principle	Multielement analysis	Applications	Other observations
Optical methods and techniques					
Atomic absorption spectrometry	AAS	Absorption of radiant energy produced by a special radiation source (lamp), by atoms in electronic ground state	Single-element technique multielement analysis (2–6 elements)	Widely used method, standard one	Most valuable technique for environmental heavy metals analysis
Inductively coupled plasma – atomic emission spectrometry	ICP –AES	Measures the optical emission from excited atoms to determine analyte concentration	Simultaneous multielement analysis	Widely used method for environmental trace analysis	
Inductively coupled plasma – mass spectrometry	ICP – MS	Argon plasma is used as ion source mass analysis is the method used for separating ions based on their mass-to-charge ratio (m/z)	Simultaneous multielement analysis	Widely used also used for isotope determination	Interferences arise when a species has the same nominal m/z as the analyte of interest
X-ray fluorescence	XRF	Uses X-rays as primary excitation source, usually provided by X-ray tubes, or radioisotopes, which cause elements in the sample to emit secondary X-rays of a characteristic λ	Simultaneous determination of most elements with the exception of those with atomic number below 8	Less suitable for analysis of minor and trace elements	Non-destructive analysis

(*continued*)

(*continued TABLE 1.*)

Method and technique	Symbol	Principle	Multielement analysis	Applications	Other observations
Neutron activation analysis	NAA	Based on conversion of stable nuclei of atoms into radioactive ones and subsequent measurement of characteristic nuclear radiation emitted by the radioactive nuclei	Simultaneous multielement analysis	Most elements can be determined with some limitations such as for Pb	Highly sensitive procedure
Atomic fluorescence spectrometry	AFS	Measures the light that is reemitted after absorption	Single-element technique	Mercury, arsenic, and selenium	Complementary technique to AAS
Molecular absorption spectrometry (colorimetry)		Relationship between molecular absorption of UV-VIS radiation by a solution and the concentration of the coloured species in solution	Single-element technique	Speciation analysis	Poor selectivity requires prior separation of the element to be determined
Separation methods and techniques					
Gas chromatography	GC	Based on the different repartition of the analyte between a stationary phase and a mobile one (gas)	Simultaneous multielement analysis	Volatile or thermally stable compounds (Hg, Sn, Pb-alkyl compounds)	Hyphenated techniques for metal speciation: GC-AAS, GC-AES, GC-MS
Liquid chromatography	LC	Based on the different repartition of the analyte between a stationary phase and a mobile one (liquid)	Simultaneous multielement analysis	Environmental metal speciation (Cr, As, Se, Sn, Hg, and Pb)	Hyphenated techniques for metal speciation:, HPLC-AAS, HPLC-AES, HPLC-ICP-AES, HPLC-ICP-MS
Ion chromatography	IC	IC is a liquid chromatography technique which uses ion-exchange resins (as stationary phase) to separate atomic/molecular ions based on their interaction with the resin	Simultaneous multielement analysis	U.S. EPA method 218.6 describes the procedure for Cr(VI) determination in water	No selectivity control hyphenated techniques for metal speciation: IC-AAS, IC-ICP-AES, IC-ICP-MS

Capillary electrophoresis	CE	Differential migration of charged analytes along a capillary filled with a suitable electrolyte	Simultaneous multielement analysis	Ions, organic and inorganic compounds of the same metal, oxoanions of As, Se	Hyphenated techniques CE-MS, CE-ICP-MS
Electrochemical methods and techniques					
Electrochemical methods		Controlled variable: voltage or current polarography potentiometry voltammetry, anodic stripping voltammetry (ASV)	Consecutive analysis of distinct metal ions is possible	Speciation analysis for transition metals and metalloids	ASV advantage: select between different oxidation states of the same metal
Biochemical methods					
Immuno chemical methods		Relies on an antibody that is developed to have a high degree of sensitivity to the target compound	Single element technique	Applicable to any pollutant for which a suitable antibody can be generated	Highly selective and sensitive

As presented in Table 1, the properties of the heavy metals valorised for their determination are as follows:

- optical: light absorption or emission spectrometry, spectrometry and colorimetry, fluorescence spectrometry and neutron activation analysis;
- repartition between a mobile and a stationary phase: gas chromatography, liquid chromatography, ion chromatography;
- migration in electrolytes: capillary electrophoresis;
- electrochemical properties, measuring the voltage or current intensity: voltammetry (anodic stripping voltammetry), polarography, potentiometry;
- specific interactions between the body-antibody complexes: immunochemical methods.

All these methods and techniques are used to determine metallic and metalloid compounds, present in different forms of a large variety of matrixes, such as environmental or biological samples. Specific properties of the methods should be emphasised, in order to highlight the criteria to be used when choosing one or another of the methods to characterise a specific sample, apart from the method sensitivity.

Some of the methods are especially applicable as single-element techniques (AFS, spectrometry and colorimetry, immuno-chemical methods), or as simultaneous multielement analyses (ICP –AES, ICP –MS, NAA, GC, LC, IC, CE).

This characteristic turns out to be an advantage or disadvantage, depending on the sample content and the analytical aim. Some of the methods and techniques are suitable to determine the cationic or oxoanionic forms of the heavy metal, and others for the organometallic forms. Speciation analysis of different oxidation states or chemical forms of a particular element (metal) is also possible. Speciation analysis is important due to the fact that the concentration of one toxic species is more relevant in the setting of environmental and ecological standards than is the total elemental concentration.

For the assessment of the environmental and human health quality, analytical techniques are subject to regulation. International organisations like United States Environmental Protection Agency (USEPA), European Environment Agency (EEA), World Health Organization Protection of the Human Environment, or national related structures are responsible for the development of directives, laws, standards, protocols and other regulations, that establish both the acceptable limits of pollutants in different matrixes and the sampling and analytical protocols for pollutant determinations.

Specialised laboratories are preoccupied to demonstrate their capability to give reliable analytical results, applying the principles of metrology in chemistry. These principles are based on validation procedures, uncertainty estimation, traceability demonstration, reference materials (RM) and certified reference materials (CRM), proficiency testing schemes (PT) – for the methods, and inter-laboratory comparisons (ILC) – for the laboratory. They are using one of the standardized analytical methods, if available, or they are entitled to use other techniques, after following a validation and recognition procedures.

3. Method Validation for Copper Cations Determination in Aqueous Samples

Validation techniques are more and more imposed by standards and specific procedures, and include different stages to demonstrate the following quality and performances criteria of the method/technique: selectivity, linearity domain of the concentration; limit of detection (LOD) and limit of quantitation (LOQ), precision (by repeatability, interim precision and reproducibility), accuracy (by recovery tests).

The experimental part of the study is aiming to give a method validation procedure for Cu^{2+} cations determination from wastewaters. The chosen analytical method was atomic absorption spectrometry with flame atomization (FAAS), not only because it is the most valuable technique for environmental heavy metals analysis, and a standard one, but also because the Romanian standard is based on FAAS procedure. SR ISO 8288/2001 standard (Determination of the content of Zn^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, from wastewaters) was used. The concentration domain imposed for Cu^{2+} is 0.05–6 mg/L. Data processing was carried out mainly using the SR ISO 8466-1/1999 (Water quality. Analytical methods assessment and calibration, performances characteristics estimation. Part I: statistic evaluation of the linear calibration function).

3.1. REAGENTS AND EQUIPMENT

The atomic absorption spectrometer GBC Avanta Σ equipped with flame atomization was used for measurements. The instrument is equipped with a deuterium lamp background correction system. Copper was determined by the flame atomization technique with an acetylene-air flame. All the reagents were of analytical purity and solutions were freshly prepared in MilliQ water.

3.2. SELECTIVITY

Even if the FAAS technique has slight interferences (Gonzales-Soto et al., (2000), a selectivity test was done, checking for the possible interferences provided by other heavy metals cations, like Cd^{2+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Fe^{2+} and Pb^{2+}. The minimal optimal concentrations (mg/L), as given in the equipment documentation, were used to prepare the testing solutions. Concentrations of Cu^{2+} as well as the absorbance were determined for 5 replicates ($n = 5$) (SR ISO 8288/2001, 2001). Due to the fact that all the registered concentrations were equal to zero, further evaluation considered the absorbance values.

In order to assess whether there are significant differences between the blank solution and each possible interfering cationic solutions, F tests were calculated as ratios of the variances (s^2) of the two pairs of data sets (blank with each cationic solution), according to equation (1), the denominator and the numerator being arbitrary, so that $F > 1$ (Uzoukwu, 2000). The results are listed in Table 2.

$$F = (s_{\text{blank}})^2/(s_{\text{cation}})^2 \tag{1}$$

Calculated F values were compared with the F-critical (tabled ones), for a confidence levels of 90% and 95%, respectively: $F_{90;5;5} = 4.11$, $F_{95;5;5} = 6.39$. All the calculated F values are lower than the F-critical ones, both for a confidence level of 90% and for 95%, showing that there are no significant differences in the registered absorbance. This means that the studied cations, possible to be present in the wastewater samples are not interfering with Cu^{2+}, and the AAS technique is selective for Cu^{2+} determinations.

TABLE 2. Selectivity test by mean of F-critical evaluation

Solutions of a possible interfering cation	Minimal optimal concentration (mg/L)	Average absorbance 10^3 (n = 5)	Variance 10^8	Calculated F values
blank	0.00	0.30	4.0	
Cd^{2+}	0.2	0.34	3.3	1.21
Zn^{2+}	0.4	2.10	1.5	2.67
Ni^{2+}	1.8	1.12	3.7	1.08
Cr^{3+}	2.0	0.50	8.5	2.13
Fe^{2+}	2.0	0.10	8.5	2.13
Pb^{2+}	2.5	2.14	1.3	3.08

3.3. LINEARITY DOMAIN OF CONCENTRATION, LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The linearity domain was tested for the concentration interval of 0.05–2.5 mg/L, with the lower limit imposed by SR ISO 8288/2001 standard and the upper limit fixed based on the laboratory experience, that Cu^{2+} in wastewaters is not exceeding 2.5 mg/L. The calibration curve, absorbance (OD) as a function of concentration, is plotted in Figure 1.

The linearity of the calibration curve is given by the $y=bx-a$ equation, where the calculated blank sample absorbance is $a = -0.0003$, the method sensitivity (the slope) is $b = 0.1089$ and the correlation coefficient is $R^2 = 1$.

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using equations (2–4) (EURACHEM guide, 1998) using the determined absorbance values for 10 replicates of blank solution, then transformed in concentration values, in order to be compared with the data obtained from the calibration curve. The results are given in Table 3.

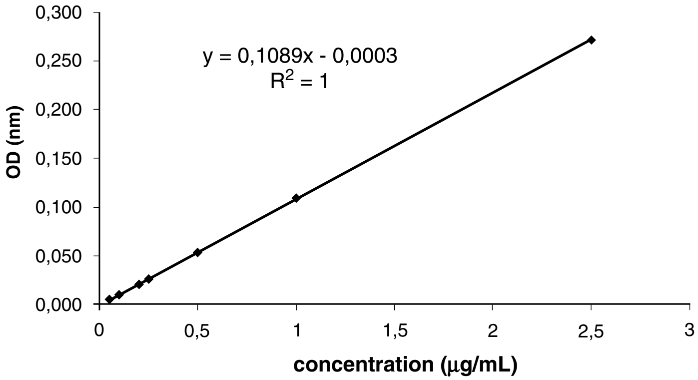

Figure 1. Calibration curve for Cu^{2+} determination by AAS technique.

$$LOD = \overline{x_{blanck}} + 3 \cdot s_{blank} \qquad (2)$$

$$LOQ = \overline{x_{blanck}} + 6 \cdot s_{blank} \qquad (3)$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \overline{x_{blank}}\right)^2}{n-1}} \qquad (4)$$

were: $\overline{x_{blank}}$ – average absorbance obtained with the blank solutions; s_{blank} – standard deviations of the blank solutions; x_i – individual discrete values of the blank solutions; n – number of replicates.

TABLE 3. LOD and LOQ for the Cu^{2+} determination by ASS technique.

LOD/LOQ	Absorbance of blank solution	Concentration of blank solution (mg/L)
LOD	0.000309	0.003
LOQ	0.000999	0.01

It is important to point out that the determined LOD value, expressed in absorbance units (0.000309) is the same with the calculated blank sample absorbance (0.0003) determined from the calibration curve. Moreover, LOD and LOQ values are lower than the imposed concentration of Cu^{2+} in waste-waters by SR ISO 8288/2001 standard (0.5 mg/L).

3.4. PRECISION

A standard solution of Cu^{2+} 0.1 mg/L was prepared ($[Cu^{2+}]_t$), repeatability was tested for 10 measurements and interim precision was tested for 10 replicates determined by three operators, each. Precision was evaluated by the relative standard deviation (RSD%), by the mean deviation (s_x) and by the confidence interval (t-distribution test) of the real concentration of Cu^{2+} ($[Cu^{2+}]_r$), for both repeatability and interim precision measurements, according to the equations (5–7) (EURACHEM guide, 1998). The results are given in Table 4.

$$RSD = \frac{s}{\overline{x}} \cdot 100 \qquad (5)$$

$$s_x = \frac{s}{\sqrt{n}} \qquad (6)$$

$$\overline{x} \pm t \cdot s_x \qquad (7)$$

were: RSD% – relative standard deviation; s – standard deviations of the tested solutions; $\overline{x}$ – average absorbance obtained with the blank solutions; s_x – mean deviation, n – number of replicates, t – t-distribution.

TABLE 4. Method precision for the Cu^{2+} determination by ASS technique.

$[Cu^{2+}]_t$ (mg/L)	n	Average $[Cu^{2+}]$ (mg/L)	Precision	RSD (%)	s_x	Final result	
						$[Cu^{2+}]_r$ (mg/L)	Confidence interval (t=2)
0.1	10	0.0984	repeatability	3.26	0.001013	0.0984 ±	0.0020
0.1	3x10	0.1014	interim precision	5.42	0.001739	0.1014 ±	0.0035

The obtained results show good precision for both parameters, and, as expected, the RSD% for repeatability is lower than the RSD% for interim precision. The mean deviation is very low and the final result may be expressed as concentration together with the confidence interval.

3.5. ACCURACY

Accuracy was evaluated by recovery tests, according to equation (8) (EURACHEM guide, 1998). A method is considered accurate if the recovery test gives values in the range of 85% ≤ R ≤ 105%. The results are presented in Table 5.

$$R\% = \frac{C_F - C_U}{C_A}100 \tag{8}$$

where: C_U – concentration determined in unfortified sample; C_A – concentration of fortification (added solution); C_F – concentration determined in fortified sample.

TABLE 5. Accuracy test for Cu^{2+} determination by AAS technique

Volume (mL)	C_U (mg/L)	C_A (mg/L)	C_F (mg/L)	R (%)
0.1	0.05	0.05	0.1035	107.0
0.3	0.05	0.15	0.2027	101.8
0.5	0.05	0.25	0.3031	101.2

The R value higher than the accepted upper limit 105% (107.0) shows that the method is less accurate for very low volumes of tested solutions, and possible errors are introduced from pipetting at such low solutions volumes (0.1 mL).

3.6. UNCERTAINTY ESTIMATION

Uncertainty assessment is one of the criteria to evaluate method performances. In order to estimate the uncertainty, the measured was specified as being the concentration of Cu^{2+} in wastewaters, the uncertainty sources were identified (Ishikawa diagram) and the different types of uncertainty were calculated according to equations (9–12): standard uncertainty (u_x); relative standard uncertainty (u_r); combined relative standard uncertainty (u_c); extended standard uncertainty (U) (EURACHEM Guide, 2000).

The final result is announced as a combination between the average concentration (C) and the uncertainty contribution (R=C±U).

$$u_x = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \overline{x}\right)^2}{n-1}} \tag{9}$$

$$u_r = \frac{u_x}{x} \tag{10}$$

$$u_c = \sqrt{\sum u_r^2} \tag{11}$$

$$U = u_c \cdot k \cdot 100 \tag{12}$$

For three standard solutions of Cu^{2+} in a range of 0.05–0.75 mg/L the calibration curve and the repeatability of the concentration were found to be the uncertainty sources, and the Ishikawa diagram was developed, as presented in Figure 2.

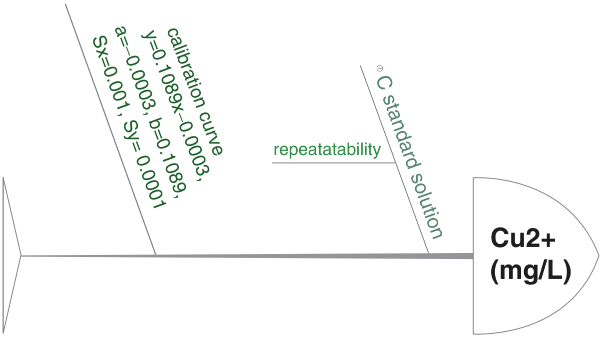

Figure 2. Ishikawa diagram for the uncertainty sources on standard solutions.

For three wastewater samples containing Cu^{2+}, the following were identified to be the uncertainty sources: the calibration curve; the repeatability of the volume of HCl used for sampling (V_1) as well as the wastewater volume (V_2). With these four contributions to the total uncertainty, Ishikawa diagram was developed, as presented in Figure 3.

According to the Ishikawa diagrams, the four types of uncertainty were calculated, with the equations (9–12), while equation (11) was adapted to the type of solutions, with equation (13), for standard solution and equation (14), for wastewater samples, respectively.

$$u_c = \sqrt{(u_r calibr)^2 + (u_r conc)^2} \tag{13}$$

$$u_c = \sqrt{(u_r V_1)^2 + (u_r V_2)^2 + (u_r calibr)^2 + (u_r conc)^2} \tag{14}$$

Extended standard uncertainty were calculated with k = 2, considering a confidence level of P = 95%. For both standard and wastewater, 10 replicates were measured and the averages concentrations were considered. The final result report will be accordingly with the Table 6.

There is a relationship between repeatability (RSD_r%), interim precision (RSD_{ip}%) and extended standard uncertainty (U%): (RSD_r%) < (RSD_{ip}%) < (U%). Comparing the results given in Table 4, where $RSD_r\% = 3.26$ and $RSD_{ip}\% = 5.42$, the above mentioned relationship is kept for the uncertainty also, with the exception of extended standard uncertainty estimated for the standard solution of 0.75 mg/L (U% = 1.51).

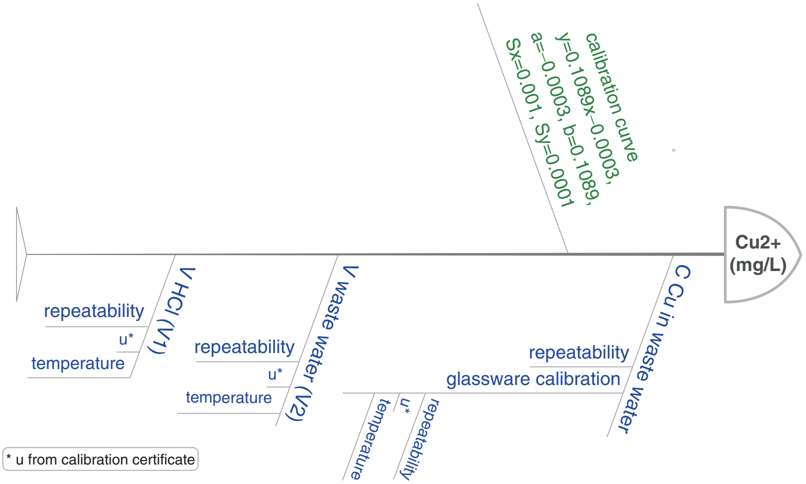

Figure 3. Ishikawa diagram for the uncertainty sources on wastewater samples.

TABLE 6. Uncertainty estimation for standard solution and wastewater samples.

$[Cu^{2+}]$ standard (mg/L)	U standard (%)	$[Cu^{2+}]$ wastewater (mg/L)	U wastewater (%)	Final result (mg/L)
0.050	7.84	0.061	8.85	0.061 ± 0.0885
0.098	6.76	0.249	8.66	0.249 ± 0.0866
0.748	1.51	1.032	8.19	1.032 ± 0.819

Considering separately the two groups of standard solution and the wastewater samples, the extended standard uncertainties are decreasing with the increase of Cu^{2+} concentration in the sample.

Moreover, for all the studied concentrations the extended standard uncertainties obtained for the standard solutions are lower than the extended standard uncertainties obtained for wastewater samples. This can be explained with the contribution brought by the sample preparation of the wastewater.

4. Conclusions

The paper presented an overview of the analytical methods and techniques available for the heavy metals determination in environmental and biological samples. The methods were grouped based on their ability to valorise specific properties of the analyte, like optical properties, repartition between a mobile and a stationary phase, migration in electrolytes, electrochemical properties, or immunochemical ones.

Due to the fact that AAS is the most used and standardized method for heavy metals concentration, a validation procedure for Cu^{2+} determination in wastewaters samples is presented. The method is selective, linear in the required range of concentration, sensitive, precise and accurate.

The uncertainty was estimated for Cu^{2+} determination in standard solution as well as in wastewater samples and the results emphasise the contribution of the sample preparation to the extended standard uncertainty.

Based on the validation procedure and the uncertainty estimations, one can conclude that the specialized laboratory is able to deliver reliable results for Cu^{2+} determination in municipal wastewaters.

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ANALYSES OF HEAVY METAL MIXTURES IN THE ENVIRONMENTAL MEDIA

LUBOMIR SIMEONOV[1]*, BIANA SIMEONOVA[2]
AND CHRISTOPH SCHMITT[3]
[1] *Solar-Terrestrial Influences Laboratory*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str. Bl.3, 1113 Sofia, Bulgaria
[2] *Institute of Electronics*
Bulgarian Academy of Sciences
72 Tzarigradsko Shossee Bul, 1784 Sofia, Bulgaria
[3] *Dr. Schmitt-ISOTOP*
Laboratory for Laser Analytical Research
Sauerwiesen 6, 67661 Kaiserslautern-Siegelbach, Germany

Abstract. The identification and measurement of heavy metals in samples of environmental origin as soil, water, ambient air or from biological objects, is performed at present by different analytical methods and techniques normally in special laboratory conditions. In cases when an express in-field chemical analysis and interpretation of the results is needed, the usual procedures, which assure the quality control of measurement and data collection, are time limited and simplified. The aim is to develop and introduce in general practice reliable methods and techniques for express elemental and isotopic analysis of the environmental media pollution, which would fulfill the measurements at acceptable quality level and would not sacrifice the quality objectives of the analysis because of compromises reasons. A presentation is made here of a small and portable laser mass-spectrometer, whose analytical capabilities are believed to fulfill the requirements for in-field chemical analysis of environmental pollution with heavy metals.

Keywords: Heavy metals, environmental pollution, in-field analysis, laser mass spectrometry

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* To whom correspondence should be addressed: simeonov2006@abv.bg

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1. Introduction

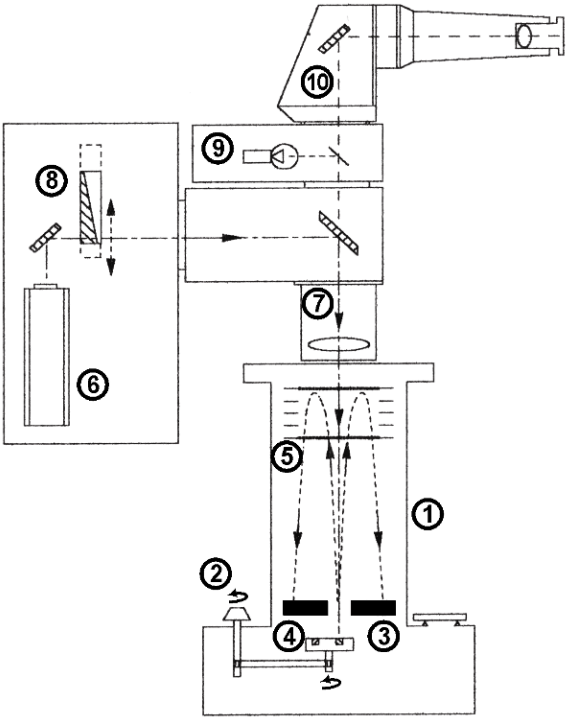
Generally the chemical measurement of the presence of heavy metals in the environment includes initially sample procedure actions as field sample taking, collection and handling. On the next step come the procedures of the actual chemical analysis of the samples and the data processing, analysis and interpretation of results. Every action on this road is carried with measures and protocols, which would validate quality assurance and quality control and address its main parameters as precision, accuracy, representativeness, completeness and comparability.

In cases when heavy metal pollution of the environmental media is a result of industrial accidents or is caused intentionally by some criminal action, or even is a case of terroristic action, there is a need of emergency and express analysis of the pollution in order to identify the scope and the reasons of occurrence, to possibly predict the speed and direction of spread, and finally to trigger if necessary some action plan to protect the people in the region of risk.

One of the possible methods of measurement and the relevant technique, appropriate to fulfill the upper stated research tasks, is the laser time-of-flight mass-spectrometric method and the developed initially for space research portable laser mass analyzer, called LASMA – **LAS**er **M**ass **A**nalyser (Schmitt, 1994; Managadze and Managadze, 1999; Woll et al., 1999; Schmitt and Schmiedel, 2007; Simeonov et al., 2005). Inventor and scientific promoter of the compact laser mass analyzer is Prof. Georgy Managadze from the Space Research Institute of the Russian Academy of Sciences. The technical director of the development is Dr. Christoff Schmitt from Dr. Schmitt-ISOTOP, Laboratory for Laser Analytical Research, Kaiserslautern-Siegelbach, Germany.

2. Instrumental Design and Main Functional Units of the Laser Mass Spectrometer LASMA

The laser-mass-spectrometer LASMA, whose schematic drawing is shown on Figure 1 consists from the following main functional units: the vacuum chamber 1 with the vacuum pump unit, providing 5×10^{-6} mbar; the target insertion system 2 with the sample holder 4; the Nd-YAG laser 6 with the beam optical and focusing system 7, beam power attenuation filter 8; the microscope 10 and the target illuminator 9; the two-stage electrostatical reflector or the reflectron; and the microchannel particle detector 3. The spectrometer has also a a power supply unit and an automatic data processing unit, which includes a transient recorder with a sampling rate of 100 MHz, a laptop computer and a printer. The whole system with the vacuum unit, the power supply and the personal computer can be mounted on a small table in the laboratory or on a rack of a medium-sized pick-up vehicle.



1. Vacuum chamber
2. Target system
3. MCP detector
4. Target holder
5. Reflectron
6. Nd-YAG laser
7. Focusing lens
8. Filter
9. Target illuminator
10. Microscope

Figure 1. Functional scheme-view of the laser mass spectrometer (Schmitt, 1994; Schmitt and Schmiedel, 2007; Simeonov et al., 2005).

The target insertion system allows the replacement of the sample holder within one minute without a need to air the whole vacuum volume of the analyzer. The sample holder is designed to carry up to ten individual samples.

The positioning of the sample is achieved by a precision vacuum micrometer screw and could be observed and controlled by the microscope system or by a video camera.

The pulsed laser beam with specific density of 10^9 W.cm^{-2} can be focused on the surface of the sample under investigation on a 20–50 μm diameter spot. The area within this diameter is heated and evaporated and ionized. The ions of different masses expand in a free flight and are reflected in the reflector 5. After reflection they meet the detector surface in different time intervals depending on their mass/charge. The detector signals are stored in the computer memory with the help of the transient recorder.

The featured physical an analytical characteristics of the analyzer, established experimentally, are the following: mass range, – 1–250 a.m.u.; mass resolution at FMHM – not less than 200; maximum resolution in individual spectra of a series up to − 600; relative sensitivity of one analysis (a single laser shot/mass spectrum) – 10^{-5}–10^{-6}; relative sensitivity in the spectrum accumulation mode – 10^{-6}–10^{-7}; absolute detection threshold in terms of mass in the single laser shot/mass spectrum mode – 5×10^{-13}–5×10^{-14} g; absolute detection threshold in the spectrum accumulation mode – 5×10^{-16} g; diameter of the sampling area – 20–50 μm; depth resolution during at layer-by-layer analysis depending on the target material – 2–3 μm; depth of the layer-by-layer analysis at one point (without shifting of the laser beam) – not less than 1 mm; reproducibility of the silver isotope ratio, measured – 0.1. The main technical characteristics of the instrument are: type of the laser – a pulsed NdYAG at wavelength of 1,064 nm; specific power density of the laser emission – 10^9 W cm^{-2}; duration of the laser impulse – 5 ns; instrument speed of response – 200 ns; overall size of the system with included vacuum and pumping-out unit, power supply and data processing units – 100 × 60 × 70 cm; weight – not more than 50 kg; power consumption – not higher than 0.6 kW.

3. Overview of Possible Application Areas of the Analyzer

The general application of the laser mass analyzer, according to the above specified physical and analytical characteristics comprise areas, which provide laser targets of different origin and material state: solid or power substances (metals, alloys, non-metals, isolation materials, ceramics, mineralogical samples, plastics, glass, vacuum persistent biological material, vaporized water and liquid solution samples, sedimentation samples, etc.

The possibility to analyze sample targets from the list above defines several application areas and among them such as *ecology*: determination of substances which pollute the environment and the food chain, by compounds of heavy metals, rare-earth elements, phosphorus, and halogenated organics in amounts not higher than the maximum permissible concentration of these compounds; in *space research*: the study of the chemical composition of the soil of planets, planetary satellites and asteroids as well as the study of materials obtained on board of the long-term orbital stations in weightlessness conditions; in *geology*: determination of the elemental and isotopic abundances in geological samples either in the field or in laboratory, the detection of elements like Li, B, S, Cl, Sc, Rb, In, Eu, Er that are difficult to be recorded with spectrometry, determination of the heterogeneous structure of samples over the surface and in depth; in *metallurgy*: a quick-look analysis of the composition of various alloys, microanalysis, determination of admixtures, control of pure materials, lateral and layer-by-layer analysis of alloyed surfaces, determination of the alloyed coatings thickness and surface homogeneity; in *microelectronics* and *electronic industry*: analysis of the chemical composition of semiconductors, determination of the value and the depth distribution of implanted ions, a quick-look analysis of the purity and homogeneity of semiconductor materials in the process of production; in *biology* and *medicine*: study of the biochemical laws for the distribution of elements in a living organism, measurements of the load on organisms for example with heavy metals and other elements with toxic action alongside with the investigation of the elemental abundances in samples, while in principle it is technically possible to implement a local freezing of the biological samples without to disturb the vacuum in the analyzer itself; in *pharmaceutical production*: continuous quick-look control of manufacturing of drugs, contamination of the materials as a result of accidental abrasion or from other pollution sources, control of the homogeneity of mixtures; in *technology*: monitoring of a technological process at different stages, study of the chemical composition of new materials.

The other possible fields of application of the compact laser mass spectrometer include: in criminality as a reliable test portable instrument for narcotics and related substances, architecture, numismatics, agro chemistry, food industry, paleontology and numerous other fields of national economy and science and technology that require the chemical analysis of various materials.

4. Discussion of the Analytical Capabilities

4.1. MASS RESOLUTION

According to the physical characteristics of the analyzer, listed above, the mass resolution can reach in certain individual cases up to 600 M/ΔM at FWHM. However the typical mass resolution, estimated experimentally with samples without any significant and time-consuming preliminary preparation of the laser targets and according to the instrument passport, is not less than 200. The mass resolution of the LASMA spectrometer is limited by its instrumental properties as well by the physical properties of the investigated samples and also by the evaporated by every laser shot material quantity. The tilt of the surface plane of the laser target with respect to its orientation to the direction of ion exit from the sample also seems to play a certain minor role in deteriorating the mass resolution. For the purposes of express in-field investigation of environmental samples an analyzer mass resolution is fairly enough.

4.2. REPEATABILITY OF MESSUREMENT

The reproducibility of the exact measurement, achevable with the compact laser mass spectrometer has been approved in a set of experiments with targets of high homogeneity. Investigated was a metal wire used for the production of the contacts of integrated circuits. A total of 56 individual laser shots were produced on the target in a sequence and the spectra data for each single laser shot was introduced automatically in the computer.

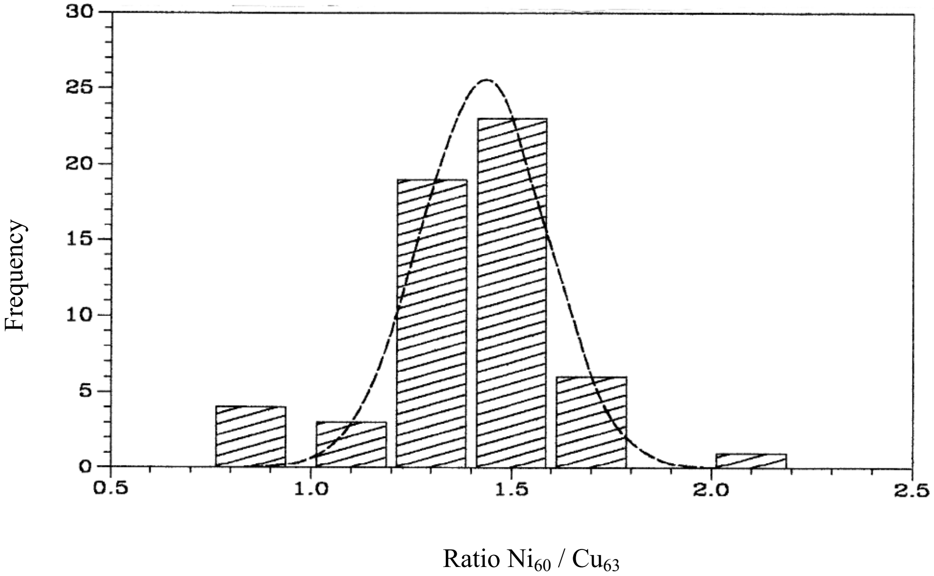


Figure 2. The frequency of measurements of the ratios Ni_{60}/Cu_{63} over the distribution of the ratios from a sequence of 56 automatic laser shots on a homogeneous NiCu metal wire (Schmitt, 1994; Simeonov and Managadze, 2006).

The spectra were produced in equal time intervals, defined and controlled in order to avoid possible false data record from unintentional laser self-shot. In the following data analysis the dependence of the computed by the program for mass identification ratios of areas Ni_{60}/Cu_{63} are plotted against the distribution of these ratios from the 56 individual laser shots (Figure 2). The Gauss-approximation shows a deviation of 12%. Assuming that the laser target is of high homogeneity of distribution of Ni and Cu, it can be stated that the measurement results have a statistical error of 12%. This error is caused by the statistical fluctuations in the production of Ni and Cu ions in dependence in dependence to the self fluctuations of the laser beam power density, which is not lower than 8% (Schmitt, 1994; Simeonov and Managadze, 2006). The reproducibility of mass spectra was checked in a similar experiment with a target of a standard Cu wire determining the characteristic isotopic ratio of Cu_{63}/Cu_{65}. The laser produced totally a series of 165 consecutive shots and the isotope ratio was calculated for every shot by the areas under the mass peaks. The averaged value of the so calculated isotope ratios was estimated to be 2.24 ± 6%, which fully corresponds to the natural abundance distribution of Cu isotopes of 2.24. Similar measurements with a standard with a standard reference material NIST SRM 610 from the United States National Bureau of Standards showed an average value of 2.24 ± 1% from 50 consecutive laser shots/mass spectra. This result shows that the small differences in the two measurements are a function of the different homogeneity of the laser targets. Further experiments with the glass reference aimed the evaluation of the reproducibility of the laser mass analyzer in the upper mass range of the heaviest elements Pb, Th and U_{238} and several additional mass peaks, which correspond to UO, ThO, UO_2 and ThO_2. Evaluated were mass spectra from 19 consecutive laser shots, each on a different spot of the matrix. The areas under every mass peak were calculated and the ratios of Th/U and Th/Pb were estimated. It was found that the spreads were respectively 13% and 26% (Schmitt, 1994; Schmitt and Schmiedel, 2007; Simeonov et al., 2005; Simeonov and Managadze, 2006), which proves an acceptable measurement reproducibility of the instrument in the region under 50 ppm elemental concentrations in the laser target.

4.3. THE INSTRUMENT CALIBRATION

The calibration of every laser mass spectrometer in principle is performed with the help of special standard reference materials with high homogeneity and certified elemental and isotopic composition. The industrially produced commercial spectrometers undergo this obligatory procedure because of possible performance differences in each instrument, introduced by its main building elements. This approach is of much greater importance and must be performed with every instrumental design, because besides the necessary evaluation of the of the analytical characteristics, the checking with with standard reference materials is in fact an evaluation of the new ideas, introduced in the instrument (Managadze and Managadze, 1999).

One of the standard reference materials, used for the instrument calibration, was NIST SRM 612, a target of glass matrix material with added certified impurities of 62 elements with nominal concentration up to 500 ppm of each element. The homogeneity is guaranteed by the passport and is certified with accuracy of 2%. The elements in the mass region from 60 to 238 a.m.u. have approximately the same presence in the standard, equal to ~50 ppm. On Figure 3 is presented a fragment of a mass spectrum from a single laser shot on the glass reference standard NIST SRM 612 as the elements with masses above 60 a.m.u. are with amplified appearance for better observation.

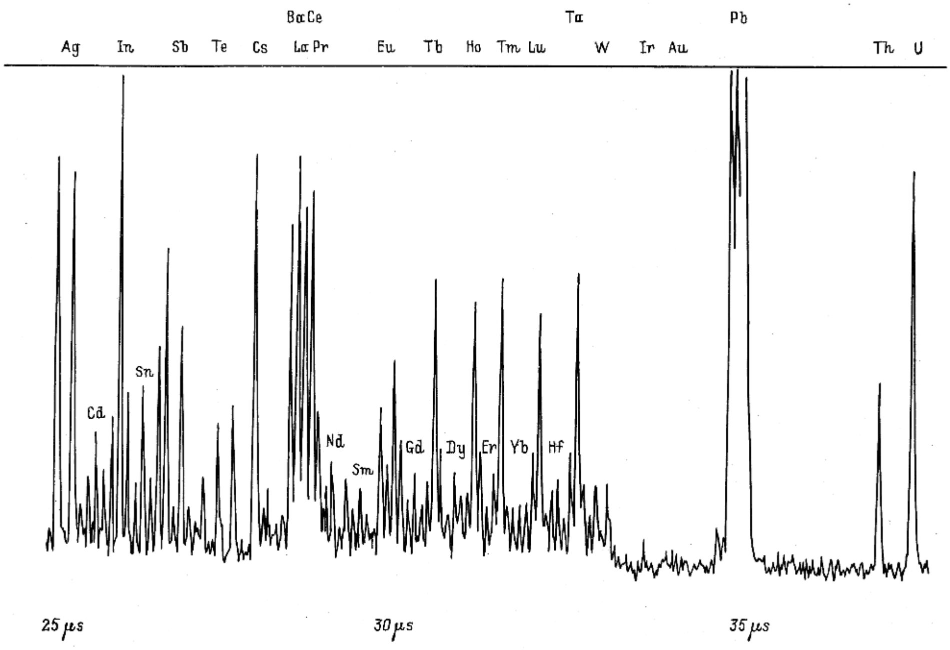


Figure 3. A fragment of a mass spectrum from a single laser shot on a glass reference standard NIST SRM 612 (Managadze and Managadze, 1999).

5. Analysis Examples

The method of laser plasma ablation has an advantage in comparison to other methods of analysis of the upper surface of electrically non-conducting materials, because the process does not load the evaporated surface. On Figures 4–8 are shown mass spectra from different materials, which are obtained by the mass analyser LASMA and produced by single shots of the laser.

On Figure 4 is shown a mass spectrum of a pulverized ceramic target obtained by a single laser shot. Generally ceramics is very difficult to analyze because of its very high melting point.

On Figure 5 is shown is shown a similar mass spectrum from a glass target. The difficulties of the measurements of laser targets of glass are very similar to those of ceramic targets. The use of laser ablation through evaporation is additionally hampered by the fact that most of the glasses are transparent to the IR laser light.

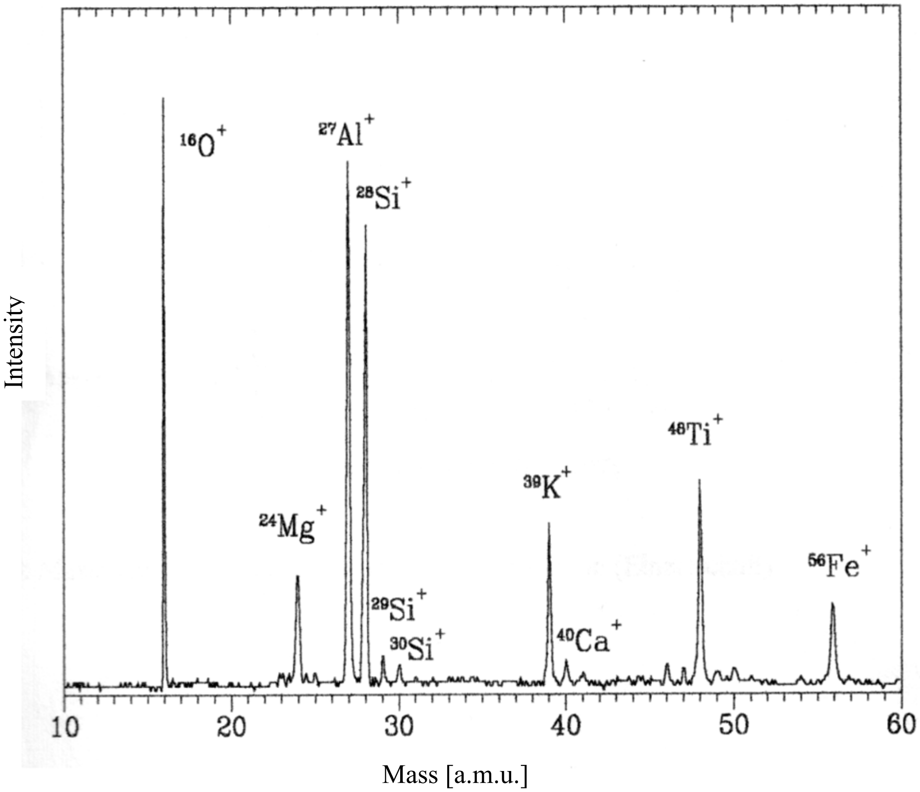


Figure 4. A single laser shot mass spectrum of a pulverized ceramic sample (Schmitt, 1994).

Previous experiments with glass targets have shown that it is possible to investigate glass targets provided that the laser light is sufficient. If the laser light is not sufficient, a possible way out that it helps is to roughen the upper surface layer of the target.

On Figures 6–8 are shown mass spectra from plastics materials. The laser ablation evaporation of such samples has the advantage that independent from the type of sample (plastics, metalloid, etc.) the material is uniformly evaporated.

On Figure 6 is presented a single laser shot mass spectrum of a plastic garbage bag.

On Figure 7 is presented a single laser shot mass spectrum of a PVC (polyvinylchloride) plastic bar.

On Figure 8 is presented a single laser shot mass spectrum of a laboratory PE – flask.

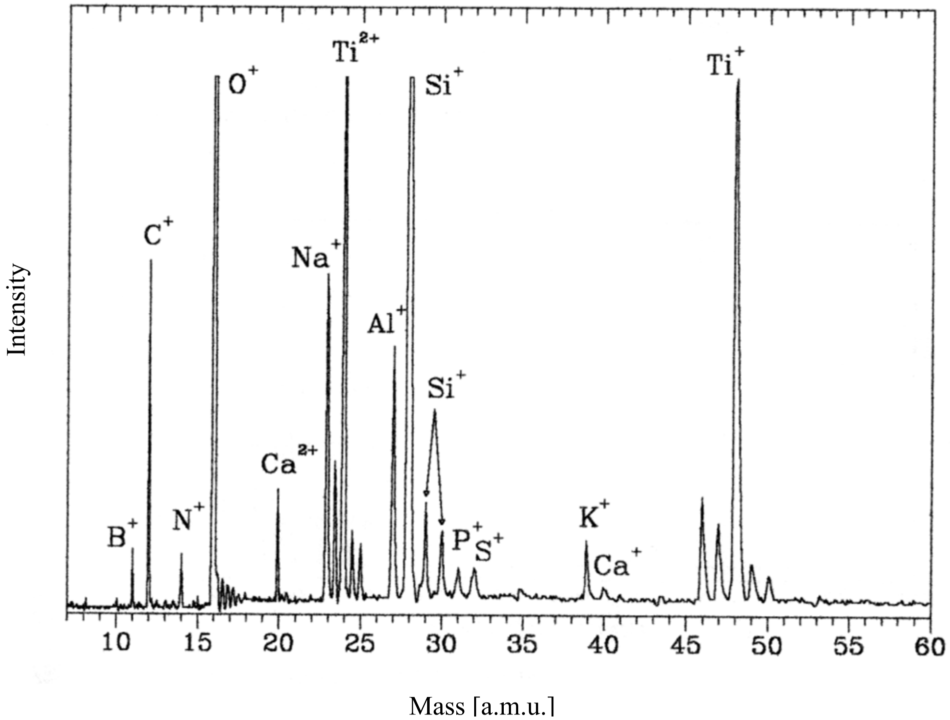


Figure 5. A single laser shot mass spectrum of a pulverized glass sample (Schmitt, 1994).

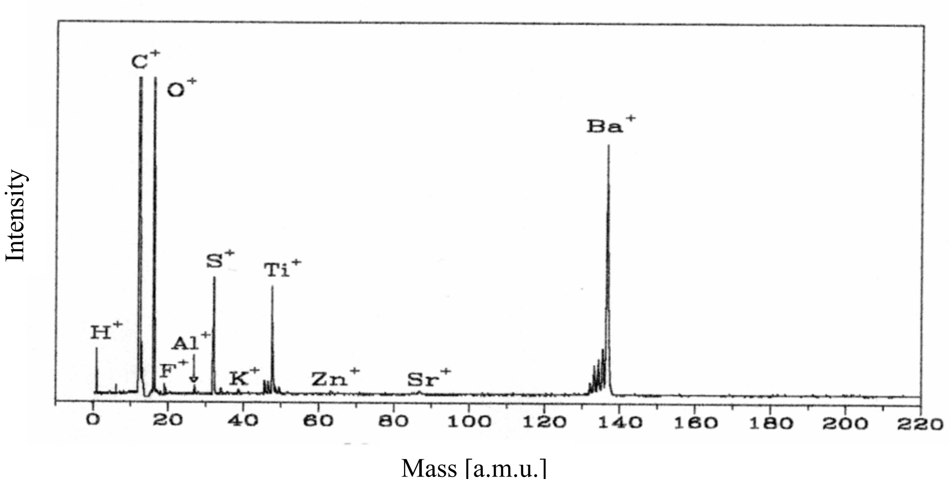


Figure 6. A single laser shot mass spectrum of a plastic garbage bag (Schmitt, 1994).

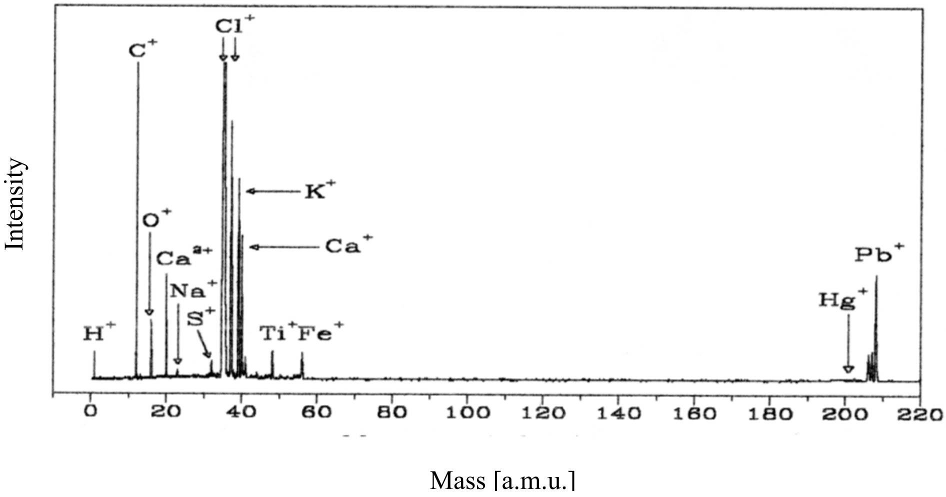


Figure 7. A single laser shot mass spectrum of a PVC plastic bar (Schmitt, 1994).

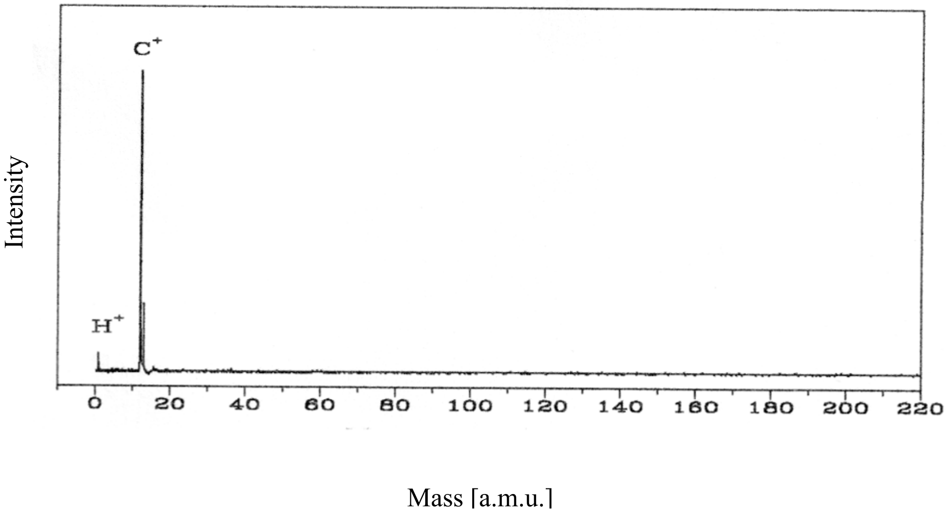


Figure 8. A single laser shot mass spectrum of a laboratory PE flask (Schmitt, 1994).

6. Conclusions

The above presented results confirm the applicability of the miniature laser mass spectrometer in different earth-based areas, where an express elemental and isotopic analysis is needed. The instrument is especially suitable for in-field environmental studies and meets the basic requirements as small dimensions and mass, high mobility, high degree of autonomy, operation in various working conditions, minimal consumption of energy and consumables, easy operation and maintenance, easy exchange of main functional units, fully automated analysis cycle, registration and identification of mass spectra.

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ATOMIC ABSORPTION SPECTROMETRY (FLAME, ELECTROTHERMAL, VAPOUR GENERATION) IN ENVIRONMENTAL, BIOLOGICAL AND FOOD ANALYSIS

DIMITER L. TSALEV*
Analytical Chemistry Department, Faculty of Chemistry
University of Sofia "St. Kliment Ohridski"
1 James Bourchier Blvd., Sofia 1164, Bulgaria

Abstract. Atomic absorption spectrometry (AAS) is one of the most valuable and well established techniques in the vast application area of biological and environmental analysis. Flame AAS is robust, selective and straightforward yet lacks sensitivity at analyte concentrations below 0.1–10 µg/g in analyses of liquid and solid samples, respectively. Two modern AAS techniques with better detection power (10–1,000-fold) as well as their combinations with each other and with other separations are applied in the lower concentration range: electrothermal AAS (ETAAS) or graphite furnace AAS (GFAAS) and vapour generation AAS (VGAAS), viz. hydride generation AAS (HGAAS) and cold vapour technique (CVAAS). Application scope and performance characteristics of these techniques are presented and some recent research and development trends are given. Illustrations by examples from the author's own research on trace element determinations (As, Bi, Cd, Hg, Pb, Sb, Se, Sn, etc.) in environmental and biological materials (water, soil, sediment, terrestrial and aquatic tissues, urine, etc.) by direct and hyphenated VG techniques, including online treatments by microwave (MW) and ultraviolet (UV) irradiation (HG–ETAAS, FI-MW–HGAAS, FI-MW–CVAAS, HPLC–UV–HGAAS) are given. An emphasis is placed on metrological and quality control issues. Review of compiled reference values on trace element concentrations in biological and environmental samples from author's own research is presented.

Keywords: Atomic absorption spectrometry (AAS), graphite furnace AAS, hydride generation AAS, cold vapour AAS, biological samples, environmental trace analysis, trace elements, speciation analysis, hyphenated techniques, sample treatment, reference values

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* To whom correspondence should be addressed: tsalev@chem.uni-sofia.bg

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1. Introduction

Modern analytical instrumentation and current methodologies provide a broad selection of analytical procedures for measurements of over 80 chemical elements within an impressive concentration spread of more than ten orders of magnitude: from macro components (>1%) down to μg/g (ppm, 10^{-4}%), ng/g (ppb, 10^{-7}%), and even to sub-pg/g (ppt, 10^{-10}%) ultra trace levels. In harmony with the role of analytical chemistry as an "in-between science" and the role of the analyst as "a problem solver" (Kellner et al., 1998), the development of technical and methodological approaches in trace elemental analysis has closely followed the needs for sensitive, selective, robust and cost-effective determination of several tens of chemical elements in the vital application field of biological and environmental area (Tsalev and Zaprianov, 1983; Tsalev, 1984, 1994, 1995; Dedina and Tsalev, 1995).

1.1. NEEDS FOR TRACE ELEMENT DETERMINATION

Up to 50 chemical elements represent interest in relation to human health and exposure (occupational, environmental, clinical, nutritional, toxicological, forensic cases, pharmaceutical, etc.), as presented in Table 1. Selected biological materials used in toxicokinetic exposure studies by means of trace element determinations are compiled in Table 2. The most important analytes and biological specimens

TABLE 1. Needs for trace element determinations in biological, environmental, food and pharmaceutical samples.

Environmental exposure: **Al, As, B, Ba, Be, Cd, Cr, Cu,** F, **Fe, Hg, Mn,** Mo, Na, Ni, **Pb,** Pd, Pt, Rh, **Sb, Se,** Si, **Sn (organotins),** Te, Ti, **Tl,** U, **V,** W, **Zn**

Occupational exposure: Ag, Al, **As**, Ba, **Be**, Bi, **Cd, Co, Cr, Cu**, F, **Hg, Mn,** Mo, **Ni, Pb, Sb**, Se, **Si**, Sn, Te, Ti, Tl, **V**, W, **Zn**

Nutrition/balance studies

Deficiency: Co, Cr, Cu, F, **Fe**, Mn, P, **Se**, Zn

Excessive levels: Ag, Al, **As**, B, Ba, **Cd**, Co, **Cr**, **Cu**, F, Fe, **Hg**, Mn, Mo, **Na**, Ni, P, **Pb**, Se, **Sn** (inorganic and **organotins**), Tl

Inert markers: Co, Cr, Yb

Clinical, toxicological and forensic interest: **Al*, As*, Au***, B, Ba, Bi*, **Ca, Cu, Fe**, Ga*, **Hg, K, Li***, **Mg, Mn, Na, P, Pb, Pt***, Rb*, **Sb*, Se, Tl*, Zn**

Pharmaceutical products

Active ingredients: Ag, **As**, Au, Bi, Ca, Co, Cr, Fe, Ge, Li, Mg, Na, Ti, Sb, Se, Zn

Impurities (e.g. from catalysts, stabilizers, preservatives, leaching from vessels, ampoules, packaging materials, etc.): Cd, Cr, Cu, Fe, **Na**, **Hg**, Ni, **Pb**, Pd, Zn

Updated from (Tsalev, 1994, 1995). Symbols in **bold** indicate major interest. *Asterisk denotes interest in abnormally high levers (toxic, therapeutic, forensic, etc.).

TABLE 2. Selected biological materials in toxicokinetic exposure studies by means of trace element determinations.

Biological material	Trace element
Bile	Cr, Mn, Pd
Blood plasma or serum	**Al**, **Au***, **Be**, **Bi***, Co, **Cr**, **Cu**, **Fe**, Ga*, **Li***, Mo, **Ni**, P, **Pt***, Se, Si, V, **Zn**
Blood (whole)	As, Be, **Cd**, Co, **Hg**, Mn, **Pb**, Sb, **Se**, Te, V, **Tl**
Blood (erythrocytes)	**Cr**, Fe, Li*
Bone	**Al**, **Pb**, Sr
Breast milk	As, Be, **Cd**, Co, **Cr**, **Cu**, **Fe**, **Hg**, **Mn**, Mo, Ni, P, **Pb**, **Se**, **Sn**, V, **Zn**
Breath (exhaled air)	Se, Te
Cerebrospinal fluid	Al, Cu, Fe, Mn, Zn
Hair or nail	Ag, Au, **As**, Ba, **Cd**, Co, Cr, Cu, Fe, **Hg**, **Mn**, Mo, **Pb**, Sb, Se, Sr, Te, **Tl**, V, Zn
Saliva	Ag, **Au***, **Li***, **Hg**, Sr, Zn
Soft tissues	Al, Cd, Cu, Fe, Ga*, Hg, Li*, Mn, Ni, Pb, Pt*, Rb, Se, Si, Zn
Sputum and nasal secretions	**Be**, Ni, **Si**
Sweat	Hg, Mn, Se, Te
Tooth	**Pb**, Sr
Urine	Ag, Al, **As**, Au*, B, Ba, Be, Bi*, **Cd**, **Co**, **Cr**, **Cu**, Fe, Ga*, **Hg**, In, Li*, **Mn**, Mo, **Ni**, P, **Pb**, Pt*, Rb, **Sb**, **Se**, Si, Sn, Sr, Te, **Tl**, **V**, Zn

Updated from (Tsalev and Zaprianov, 1983; Tsalev, 1994, 1995). Matrices and symbols in **bold** indicate major interest. *Asterisk denotes analyses at abnormally high levels (therapeutic, acute intoxication, forensic, etc.).

are indicated in an appropriate way (see more details in Tsalev and Zaprianov, 1983 and other recent texts.). Although analyses of blood, blood serum or plasma and urine are dominating, there is an interest in alternative biological specimens such as hair, (toe) nails, exhaled air, deciduous tooth, saliva, etc., for various reasons (non-invasive sampling, higher content of the element, toxicokinetic and toxicodynamic studies, forensic interest, etc.).

1.2. ANALYTICAL TECHNIQUES FOR TRACE ELEMENT DETERMINATION IN BIOLOGICAL AND ENVIRONMENTAL FIELD

Several appropriate trace elemental analysis techniques are applicable in this field: *atomic absorption spectrometry* (AAS) in several modes – flame AAS (FAAS), electrothermal AAS (ETAAS) known also as graphite furnace AAS (GFAAS), vapour generation AAS (VGAAS) divided into hydride generation

AAS (HGAAS) for the hydride forming elements (HFEs) with covalent, volatile hydrides As, Bi, Cd, Ge, Pb, Sn, Se, Sn, Te and cold vapour technique (CVAAS or CVT-AAS) for mercury, and hyphenated AAS techniques such as vapour generation with in-situ trapping hydrides in a graphite atomizer (VG–ETAAS, HG–ETAAS, CV–ETAAS), gas-chromatography–AAS (GC–AAS), high performance liquid chromatography–AAS, etc.; *atomic fluorescence spectrometry* (AFS) (mainly used for Hg and HFEs in VG mode (VG-AFS and in hyphenated techniques as VG-AFS detector), thus providing better sensitivity and LODs by an order of magnitude vs. VGAAS detection (Karadjova et al., 2005; Bohari et al., 2007; Ito et al., 2010; Chen and Belzile, 2010); *inductively coupled plasma–optical emission spectrometry* (ICP-OES) (more sensitive than FAAS for refractory elements Al, B, Ba, Mo, Os, P, Pd, Pt, REEs (lanthanoids), S, Si, Sn, Ti, Tl, Re, Rh, U, V, W, Zr, and with simultaneous multielement capabilities); *inductively coupled plasma mass spectrometry* (ICPMS), *X-ray fluorescence* (XRF), mainly in its energy dispersive mode (EDXRF); *neutron activation analysis* (NAA), preferably in its instrumental, multielement mode (INAA), and (rarer) miscellaneous classical analytical techniques such as *molecular spectrophotometry* (for Cu, Fe), *flame emission photometry* (for Li, Na, K); potentiometry with *ion selective electrodes* (ISE) (for F^- and Li^+); voltammetry with in situ enrichment such as *differential pulse anodic stripping voltammetry* (DPASV) or *differential pulse cathodic stripping voltammetry* (DPCSV); *fluorimetry* (for Se); *catalymetry*, etc. Among these analytical tools, the modern techniques for simultaneous multielement ICPMS and ICP-OES have already been installed in ca. 10,000 and 23,000 laboratories, respectively, and are extensively developing and expanding with a rate of about 1,000 instruments each per year.

There is an ever growing interest in the determination of relevant element species with higher biological/environmental relevance, the so called *speciation analysis* (Cornelis, 2005) or otherwise the analysis of certain fractions comprising of a sum of relevant species or relevant extracts from analyte species partitioning (*fractionation analysis* by means of operationally defined procedures or empiric procedures). Examples of these two kinds are the determination of the more toxic methylmercury in addition to the total mercury contents in marine tissues or sediments (review by Stoichev et al., 2006), quantification of the more toxic and carcinogenic Cr(VI) species in waters, or otherwise the determination of leachable Cd in ceramic ware, the analyses of suitable soil extracts or the determination of a ‘toxicologically relevant As’ in urine (the sum of As(III) + As(V) + monomethylarsonate MMA + dimethylarsinate DMA), so as to differentiate from the virtually non-toxic ‘fish arsenic’ (arsenobetaine, etc.) from aquatic food contribution (e.g. Guo et al., 1997; Petrov et al., 2006; Georgieva et al., 2007). Essential details on speciation analysis could be derived from recent literature (Cornelis, 2005; Welz and Sperling, 1998; Dedina and Tsalev, 1995; Tsalev, 1995; Stoichev et al., 2006). With a view to speciation analysis, vapour generation AAS and AFS techniques exhibit certain potential (Section 2.5 and review by Tsalev, 1999b), while most powerful, sensitive and versatile are the ICPMS detectors to gas chromatography (GC) and high performance liquid chromatography (HPLC) (Cornelis, 2005; Sanz-Medel, 1999).

1.3. ESSENTIAL METHODOLOGICAL CONSIDERATIONS IN TRACE ELEMENT ANALYSIS

There are several general sources of errors during sample storage, preparation, separation/enrichment (if applied), and instrumental measurement: (i) external contamination, (ii) partial or complete loss of the analyte, (iii) uncontrolled physico-chemical transformations, and (iv) interferences from matrix constituents. These potential sources of error will only be listed here, since their nature, effect and control are rather specific for individual techniques, analytes and matrices and have been detailed in numerous relevant texts and original analytical procedures (Welz and Sperling, 1998; Tsalev and Zaprianov, 1983; Tsalev, 1984, 1995; Dedina and Tsalev, 1995).

- **Contamination.** Airborne contamination from dust or vapours (Hg^0) is typical for elements that are ubiquitous and present in the Earth's crust (and soil, dust, ash, etc.) at levels exceeding by several orders of magnitude their normal concentrations in biological fluids and water samples (in the ppb (ng/g) and sub-ppb range), e.g. Al, Ca, Cr, Fe, Hg, K, La, Mg, Mn, Na, Pb, Si, Ti, V, Zn. For example, there are pronounced problems with exogenous contamination of blood serum samples at ppb levels of Al, Co, Cr, Mn, Pb, Si, V, since the concentration of these elements in the lithosphere are higher by 10^4–10^7 fold vs. their normal levels in blood serum or plasma (Tsalev, 1994). Other contamination sources could be tobacco smoke (Al, Be, Cd, K, Mg, Mn, Zn), cosmetics and jewelry (Ag, Al, Au, Bi, Ti, Zn), blood sampling devices (Co, Cr, Fe, Mn, Mo, Ni) and blood/ plasma/serum storage containers and preservatives (Al, Cd, Co, Cr, Mn, Pb, Si, Zn), laboratory reagents (Al, As, B, Bi, Cd, Cr, Cu, Mn, Ni, Pb, Se, Si, Sn, Zn), etc. Strict contamination/blank control is essential in all stages of total analytical process – from carefully planned sampling to final instrumental measurement (Tsalev, 1984, 1994, 1995).
- **Analyte losses** may occur due to the many unwanted processes: (i) volatilization during dry or wet ashing of samples: As, B, Cd, Cr, F, Ge, Hg, Pb, Sb, Se, Sn, Te, Tl, Zn; (ii) hydrolysis and sorption of hydrolyzed products on walls, tubing, pipette tips, micro precipitates,

etc.: Ag, Al, Bi, Hg, Pb, Sb, Si, Sn, Ti, W; (iii) undissolved residues: Ag, Al, Au, Ba, Be, Cr, Ir, Mo, P, Pd, Pt, Sb, Si, Sn, Sr, Ti, V; (iv) incomplete release of analyte ions upon deproteinization of blood, serum, milk, etc.: As, Au, Co, Mn, Ni, Se; (v) incompletely dissolved fat fraction, e.g. Co and Ni in milk, and other reasons. This potential source of errors is best controlled by use of matrix certified reference materials (CRM), comparison with independent established technique, adequate recovery tests and other methodological approaches (Tsalev, 1995).

- **Uncontrolled physico-chemical transformations** of the analyte are most pronounced in AAS techniques that rely on major sample preparation steps such as chemical enrichment and separation/preconcentration. In some of these techniques the analyte should not only be brought in solution as simple ions but has to be present in final solutions in a definite oxidation state: e.g. to inorganic ions of As(III), Sb(III), Se(IV) or Te(IV) in vapour generation techniques (Dedina and Tsalev, 1995).
- **Interferences** in environmental analysis are often difficult to predict. Efficient background correction and elaborated chemical modification are essentials in ETAAS, where procedures with Zeeman correction (Tsalev, 2000) and the emerging High-Resolution Continuum Source AAS (Welz et al., 2005) have demonstrated their usefulness in many difficult analytical tasks. The apparent simplicity of AAS principles and instrumental operation may well happen to be a misleading and discouraging factor. Particularly, ETAAS and HGAAS can produce not only highly sensitive, accurate and reproducible results but also artefacts (!), hence the early history of these techniques has documented such problems. It is worth noting that hydride forming species exhibit individual behaviour in VG techniques and are relying on in-situ, instantaneous chemical separations, which depend on critical chemical factors (Dedina and Tsalev, 1995; D’Ulivo, 2010). Chemical modifiers in ETAAS may well happen not to work in a straightforward manner with different chemical species and matrices (Tsalev and Slaveykova, 1998). Examples for a difficult analyte such as arsenic in marine tissues have been given in a recent paper (Karadjova et al., 2007). Discussion on mechanisms, interferences and methodology of these ‘flameless’ AAS techniques is outside the scope of this chapter but could be found in recent monographs (Welz and Sperling, 1998; Dedina and Tsalev, 1995) and reviews (Tsalev, 2000; D’Ulivo, 2010).
- **Calibration.** Proper calibration strategy by means of standards matched with prepared sample solutions by acid, solvent, diluent, additives (modifiers, spectrochemical buffers) is an essential requirement in AAS techniques. Use of advanced internal standardization approaches such as

internal standards or isotope dilution is uncommon in AAS because of single element and non-isotopic mode of these measurements, but standard additions are recommended for quality control and occasionally for calibration. Using matrix certified reference materials (CRM) for calibration is impractical and expensive but is highly recommended as quality control measure.

2. Atomic Absorption Spectrometry in Environmental, Biological and Food Analysis

2.1. GENERAL CHARACTERIZATION OF AAS TECHNIQUES

Atomic absorption spectrometry (AAS) (Welz and Sperling, 1998) is one of the most valuable techniques in the vast application area of biological and environmental analysis (Tsalev and Zaprianov, 1983; Tsalev, 1984, 1994, 1995). It is now a very well established, reliable and cost-effective analytical tool in thousands of laboratories throughout the world for quantitative determinations of chemical elements (up to 60–70 analytes) at trace levels – down to 10^{-9}–10^{-12} g. Flame AAS is robust, selective and straightforward yet lacks sensitivity at analyte concentrations below 0.1–10 µg/g in analyses of liquid and solid samples, respectively.

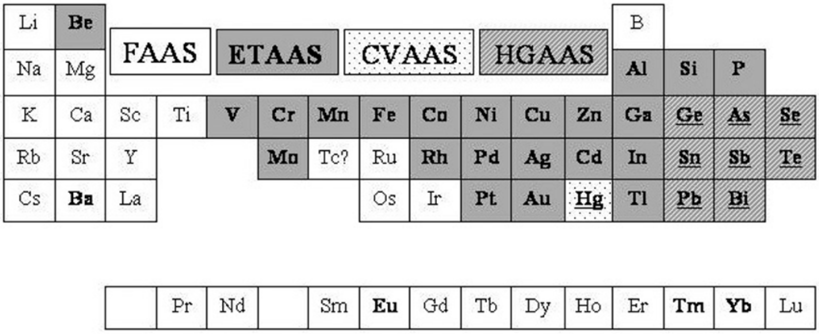

Figure 1. Elemental scope of AAS techniques: *FAAS* (no fill), *ETAAS* (shadowed field and bold symbols), *CVAAS* (dotted field), *HGAAS* (hatched fields) and both *VGAAS* and *ETAAS* techniques for elements with underlined symbols.

Two modern AAS techniques with better detection power (10–1,000 fold) as well as their combinations with each other as well as with other separations are applied in the lower concentration range: electrothermal AAS (ETAAS), also widely known as graphite furnace AAS (GFAAS) (Tsalev, 1994, 1998, 1999a) and vapour generation AAS (VGAAS) (Welz and Sperling, 1998;

Tsalev, 1999b, 2000), viz. hydride generation AAS (HGAAS) (Dedina and Tsalev, 1995) and cold vapour technique (CVAAS) (Stoichev et al., 2006). Application scope and performance characteristics of these techniques are presented and some recent research and development trends are given.

The most important performance characteristics, advantages and limitations of AAS techniques differ significantly between individual techniques, depending also on the particular analyte–matrix couple as well as on element concentration levels. As a general guide, the following main characteristics could be given in Table 3 (see also Tsalev, 1995, 1998, 2000).

TABLE 3. Ranking of AAS techniques based on their metrological and technical characteristics.

Selectivity: FAAS > CVAAS > ETAAS > HGAAS

Sensitivity:

Absolute sensitivity, dA/dm)ETAAS > CVAAS > HGAAS > FAAS

Relative sensitivity, dA/dc) CVAAS, HGAAS > ETAAS > FAAS

Automation: FAAS, ETAAS > CVAAS > FI-HGAAS, CF-HGAAS > batch HGAAS

Relatively simple sample pretreatment: FAAS, ETAAS > CVAAS > HGAAS

High sample throughput rates: FAAS > HGAAS, CVAAS > ETAAS

e.g. >150, 30–60 and 30–40 samples/hour, respectively.

Large elemental coverage: FAAS > ETAAS > HGAAS > CVAAS

e.g. 70, 50–60, 8–9 and 1–2 (Hg and Cd), respectively.

Except for the elements that cannot be determined by AAS: Ar, Br, C, Cl, F, H, He, I, Kr, N, Ne, O, Rn, S, Xe and actinoids and except for ETAAS with graphite atomizer that is not applicable to refractory Ce, Ga, Hf, La, Nb, Nd, Os, Pr, Ra, Re, Sm, Ta, Tb, Th, U, W, Y and Zr.

Small sample size requirements: ETAAS < FI-FAAS < FI-HGAAS, FI-CVAAS < FAAS < HGAAS, CVAAS

e.g. 0.01–0.05, 0.1–0.5, 0.1–2, 0.5–2 and 5–20 mL, respectively.

Adequate precision: RSD within 0.5–3% for FAAS and 2–5% for ETAAS and HGAAS

Moderately priced, affordable equipment.

Well established methodology and extensive documentation.

Limited potential for simultaneous multielement analysis: already rivalled by ICP-OES and ICP-MS.

Narrow linear working range: disadvantage in environmental and multielement analysis.

Liquid samples preferred: more conveniently homogenized, diluted, handled by autosamplers and introduced into atomizers than are solids, powders, suspensions (slurries) and emulsions; calibration is also facilitated with liquids.

ETAAS (solid microsamples and slurries possible) >> CVAAS >> FAAS > HGAAS.

Very limited potential for speciation: HGAAS > CVAAS >> ETAAS >> FAAS FAAS lacks sensitivity; ETAAS is unsuitable detector for GC and HPLC.

Individual radiation source (lamp) needed for each element (except for with high-resolution continuum source AAS, see Welz et al., 2005).

Large variety of instrumentation which is far from unification: ETAAS < HGAAS < CVAAS < FAAS.

Among the limitations and drawbacks of the AAS techniques are worth mentioning the following: (ii) AAS is as a rule a single element technique and multielement determinations are usually performed in a sequential mode, element by element – yet with some instruments in a ‘fast sequential mode’. True simultaneous determinations (4–6 elements) are possible with some instruments but at compromise conditions. In this respect AAS is seriously rivaled by techniques with true multielement capabilities such as ICP-OES and ICP-MS. (ii) AAS exhibits linear calibration range, typically within 2–3 orders of magnitude, as opposed by 10^5–10^6 and 10^5–10^8 in ICP-OES and ICP-MS, respectively. (iii) dissolution of solid samples is typically needed for most flame and HG AAS applications (see details for individual elements in Welz and Sperling, 1998; Tsalev, 1995; Dedina and Tsalev, 1995).

Sample preparation considerations

There are several basic rules for all AAS analytical methods and some specific considerations for individual AAS techniques that could be considered.

- *Rule 1:* Rational sample pretreatment in order to match requirements of particular AAS technique – FAAS, ETAAS or techniques with separation/enrichment VGAAS, CVAAS, etc.
- *Rule 2:* Simple treatments in order to avoid external contamination, losses and extensive blanks.
- *Rule 3:* Use of acid/solvent/diluent-matched standards for calibration.
- *Rule 4:* Strict quality control/quality assurance (QC/QA) measures during all steps of total analytical process. The well known selectivity of the AAS techniques should never be over trusted, particularly with new analytical tasks and poorly validated procedures.

Some more specific sample preparation considerations for individual AAS techniques are given below.

Flame AAS

- Dilute HNO_3 and HCl are best media.
- H_2SO_4 and H_3PO_4 are tolerated (but with acid-matched solutions).
- HF is corrosive (special care and corrosion resistant appliances are required; otherwise residual HF could be masked with excess of H_3BO_3 to HBF_4).
- Transport interferences are common (due to differences in viscosity, surface tension, high total dissolved contents (>0.5% m/v) and other bulk matrix effects).

- Suitable organic solvents could be beneficial for better sensitivity and dissolving (residual) lipids (e.g. 10–20% v/v ethanol or propanol; ≤10% v/v acetone; with solvent/diluent matched calibration). C_5–C_6 esters and ketones as well as xylene are suitable solvents in extraction–FAAS procedures (e.g. methylisobutylketone, MIBK and butylacetate).
- Total dissolved solids (TDS) contents in final solutions should generally be below 0.2–1% m/v although higher tolerance to TDS (1–5% m/v) could be provided in flow injection (FI) or pulse nebulization (PN) modes.

ETAAS (GFAAS)

- Dilute HNO_3 is the best medium (0.03–0.15 M, up to 1M).
- H_2SO_4, HF and $HClO_4$ are highly corrosive to graphite tubes and (less so) to pyrolytic graphite coated platforms and total pyrolytic graphite parts.
- Suitable media are EDTA, organic acids, NH_4^+ salts, tetraalkylammonium hydroxides $(CH_3)_4NOH$, $(C_2H_5)_4NOH$ (Tsalev et al., 1993).
- Organic matter is ashed *in-situ*; if needed some alternate gas (air, O_2) could be added during an intermediate step of temperature programme (e.g. Se in blood serum; analysis of fatty samples, etc).
- Chemical modification is generally required for up to 30 analytes with high and moderate volatility (see Tables 4 and 5 and extensive reviews by Tsalev et al., 1990; Tsalev and Slaveykova, 1998).
- Fast background absorbance could be problematic to correct.
- Organic solvents are generally troublesome in GFAAS (may need special solvent resistant kits and ‘hot injection’ approaches.
- Strict contamination control is essential; deionized water and high-purity reagents mandatory for efficient blank control.
- Total dissolved solids (TDS), <0.1–1% m/v, are tolerated in final solutions, strongly depending on relative volatility of matrix and analyte.
- Both slurry sampling (SS) or solid sampling (SS) are worth considering in order to facilitate sample preparation and to reduce reagent blanks.

VGAAS

- Dilute HCl is the best medium.
- H_2SO_4 and H_3PO_4 are tolerated.
- HF is corrosive and should better be avoided (special care to eliminate or mask as HBF_4 with H_3BO_3).
- pH adjustment is critical for certain analytes, especially for Cd, Pb, Sn.

- Technique exhibits high tolerance to (some) dissolved solids (e.g. to salts of alkaline and alkaline-earth elements).
- Deionized water and high-purity reagents are used throughout.
- Volatilization losses may occur during sample preparation (most pronounced risks for As, Hg, Se and Sn).
- Oxidation state and binding state effects should be controlled by proper digestion procedures and pre-reduction for As(V) → As(III), Sb(V) → Sb(III), Se(VI) → Se(IV), Te(VI) → Te(IV); non-hydride reactive species such as arsenobetaine, arsenocholine, tetramethylarsonium, arsenosugars, heavy organotins, etc. may give underestimated total contents in direct HGAAS measurements.
- Strict contamination control is essential during sample treatment (often high blanks for As, Cd, Pb, Sn, etc.).

Some economic considerations of the four major techniques in trace and ultratrace elemental analysis for the USA have been presented by Thomas (2000). The annual operating cost for an instrument running 1,000 h/year have been estimated at ca. 4,000, 5,600, 6,800 and $15,500 for the major current techniques for trace element analysis FAAS, ETAAS, ICP-OES and ICPMS, resp. (Thomas, 2000). Estimated operating costs per sample requiring ten or one analyte (in $/sample) have been 0.22, 5.60, 0.34, and $0.78 per sample for ten analytes, and 0.02, 0.56, 0.34, and $0.78 per sample for one analyte (Thomas, 2000). More conservative estimates, including sample pretreatment, calibration and quality control could multiply these figures by factors of 2–10.

2.2. FLAME ATOMIC ABSORPTION SPECTROMETRY

Flame AAS is nowadays a very well established and documented technique at ppm levels of trace elements, although it often lacks sensitivity at analyte concentrations below 0.1–10 μg/g in analyses of liquid and solid samples, resp. Attractive assets of FAAS are its remarkable selectivity, good precision, robustness, reliability, high sampling frequencies, cost-effectiveness, relatively simple optimization, availability of standardized/validated procedures. Because of lack of sensitivity in FAAS, the number of analytes is limited to alkaline and alkaline-earth metals and occasionally to some other 10–15 elements. Analytical procedures are available for water (Ca, Fe, K, Mg, Na, Zn), soil and sediments (Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Zn), blood and blood fractions (Au*, Ca, Cu, Fe, K, Li*, Mg, Na, Zn), urine (Ca, Cu*, Fe, K, Li*, Mg, Na, Zn), milk (Ca, Fe, Li*, Na, K, Mg, Zn), hair and nails (Cd, Cu, Fe, Mn, Pb, Zn), etc. (asterisk indicates applicability to abnormally high analyte levels, e.g. under Au or Li therapy).

There is an interest in decreasing sample volumes in FAAS by means of *pulse nebulization* of small sample aliquots or using *flow injection* (FI) FI-FAAS (Fang, 1993, 1995; Welz and Sperling, 1998). Both scaling-down options are commercially available, including an automated mode, thus offering increased sample throughput rates and analysis of small sample volumes or concentrates or otherwise samples with unfavorable properties for continuous nebulization. In the *pulse nebulization mode* (PN, or injection method), only 50−150 µL of sample aliquot is manually injected via a small PTFE (polytetrafluoroethylene) funnel attached to the nebulizer capillary or otherwise the autosampler probe is dipped for a preset time into solution held in (micro) sample cup. PN mode could be attractive in analyses of small samples (diluted blood or serum, etc., solubilized hair or nail, etc.) and concentrates (extracts, organic solvents, high-salt samples or digests, etc.), digests or leachates with high acid concentrations (>5−10% v/v), etc. Examples of such flame microsampling could be given: analyses of TMAH solubilized nail or hair for the determination of Cd, Cu, Fe, Mn, Pb, Zn (Tsalev et al., 1993), or chelate extraction–FAAS in microscale with sequential multielement quantification in analyses of soil extracts and plant tissues (Tsalev and Petrov, 1979; Petrov et al., 1981), etc. (see more examples in Tsalev, 1984, 1995). Noteworthy, efficient background correction is essential in PN mode of FAAS. Technique could be supplemented by involving accessories – adapters for flame gases called STAT® (Slotted Tube Atom Trap) or ACT® (Atom Concentrator Tube), which are commercially available. They could further increase sensitivity for volatile elements (As, Cd, Pb, Zn, etc.) by a factor of 2–5, owing to the increased residence time of atoms in the slotted tube (e.g. Cd and Pb in small samples of nail clippings (Tsalev et al., 1993).

2.3. ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY (GRAPHITE FURNACE AAS)

ETAAS (GFAAS) offers the lowest ILODs in the pg and ng range (Table 4) and is attractive for its applicability to intact or simply diluted liquid samples (5–50 µL), analyses of solid (<1 mg) or slurried microsamples (10–20 µL of 1–5% m/v slurry), possibilities for *in situ* treatment of interferences (Table 5) by means of efficient chemical modification (Tsalev et al., 1990), automation of instrumental measurement. The main drawbacks of this technique are relatively low sampling frequency (20–40 h^{-1}), more complex optimization and calibration, and larger expenses.

TABLE 4. Typical characteristic masses (m_o) and best instrumental limits of detection (ILOD) in ETAAS with graphite atomizer.

Analyte	*m_o (pg)*	*ILOD (pg)*	*Chemical modifier*	*Comments*
Ag	0.7–1.7	0.1–0.5	a, b, c	
Al	5–12	2–5	d	h
As	7.5–40	9–30	a, b, W, Zr	i
Au	4.4–18	10	b	i
B	500–1,000	500–2,000	a	e, g, h, i
Ba	6.5–15	5–10		f, g
Be	0.5–1	0.5–1	d	
Bi	9–28	10	a, b	i
Ca	0.5–1	5		f, g, h
Cd	0.2–1.3	0.3	c	i
Ce	8×10^4	–		e, g
Co	2.9–17	1–3	d	
Cr	0.8–3.5	0.3–1	d	h
Cs	4–18	5	d	f, g
Cu	4–17	1–2	d	f
Dy	20–140	–		e, g
Er	70–280	–		e, g
Eu	18–34	–		e, g
Fe	1.2–12	1–2	d	f, h
Ga	4.5–42	10	a, b	i
Gd	$3{,}500–1.4 \times 10^4$	–		e, g
Ge	8–34	13–20	a, b, W, Zr	i
Hg	20–220	25–100	b	g, i
Ho	61–280	–		e, g
In	7–15	5	a, b	i
Ir	135–275	200		e, g
K	0.4–1.1	2		f, g, h
La	$7{,}400–2.6 \times 10^4$	–		e, g
Li	1–4	2–5	c	f, g
Lu	52–3,500	–		e, g
Mg	0.2–0.35	0.4–0.5		f, g, h
Mn	0.6–6.3	1	d	
Mo	2–12	4–9	d	
Na	0.4–1.2	5		f, g, h
Nd	900–1,800	–		e, g
Ni	4.8–20	10	d	
Os	1,400	270		e, g
P	1,200–3,000	3,000	a, b, Zr	g, i

(*continued*)

(*continued TABLE 4.*)

Analyte	*m_o (pg)*	*ILOD (pg)*	*Chemical modifier*	*Comments*
Pb	5.5–12	5	a, b, c, W, Zr	i
Pd	8.6–52	5–25	d, W, Zr	
Pr	4,400–10^4	–		e, g
Pt	54–220	33–50		g
Rb	1–10	3–5		f, g
Rh	8–24	10		e, g
Ru	15–45	–		e, g
Sb	10–19	15–20	a, b, W, Zr	i
Sc	30–130	–		e, g
Se	20–45	10–30	a, b, W, Zr	i
Si	15–120	40	a, b	h
Sm	230–800	–		e, g
Sn	10–90	2–20	a, b, W, Zr	i
Sr	1–4	2		f, g
Tb	3.5–4,100	–		e, g
Tc	–	60		g
Te	9–17	10	a, b, W, Zr	i
Ti	32–70	50–100		e, g
Tl	10–19	10	a, b	i
Tm	13–18	–		e, g
U	1.2×10^4	–		e, g
V	13–50	3.5–48	d	g
Y	1,300–4,000	–		e, g
Yb	2.5–3.0	–		e, g
Zn	0.04–1	0.1–1	c	f, h, i

Characteristic mass (m_o) and characteristic concentration (C_o) are the mass or concentration of the analyte, respectively, that gives an analytical signal (absorbance) of A = 0.044, typically in integrated aabsorbance A_{int} (peak area measurements). Chemical modifiers: [a]Pd + $Mg(NO_3)_2$; [b]Pd, Pd + reductant; [c]PO_4^{3-}; [d]$Mg(NO_3)_2$ (see also Tsalev and Slaveykova, 1998). Comments: [e]very poor sensitivity, carryover and memory effects because of strong carbide formation; [f]flame AAS preferred in most applications; [g]ETAAS inappropriate technique; [h]external contamination and blank problems; [i]chemical modification by thermal stabilizer often required for this analyte.

In-situ chemistry is efficiently performed in ETAAS by means of chemical modification with mixed, composite and permanent modifiers for thermal stabilization of volatile analytes; isoformation and leveling-off responses of various analyte species; stabilization or elimination of interfering matrix constituents; improving modifier efficiency by means of permanent coatings (carbides, non-volatile noble metals and noble metals on carbide coatings), etc. (Tsalev, 2000; Tsalev et al., 1990, 2000a, 2002).

TABLE 5. In situ chemistry in graphite atomizer for solving problems with interferences by chemical modification (see also Tsalev et al., 1990; Tsalev and Slaveykova, 1998).

Interferences or problems	*Remedies*
Chlorides	HNO_3, NO_3^-, H_2, NH_4^+
Phosphates	Pd, Ni, $Mg(NO_3)_2$, coatings (Zr, W, V, Mo, La, etc.)
Fe	Pd, Pt, $Mg(NO_3)_2$, coatings (Nb, etc.)
Sulfur	Mixed modifiers (containing Ba, Mo, La)
$HClO_4$	TaC coating
Carbonaceous residues	*in situ* ashing with O_2 or air alternate gases
Residue build-up from non-volatile modifiers	Permanent modifiers, e.g. W−Ir, Zr−Ir etc

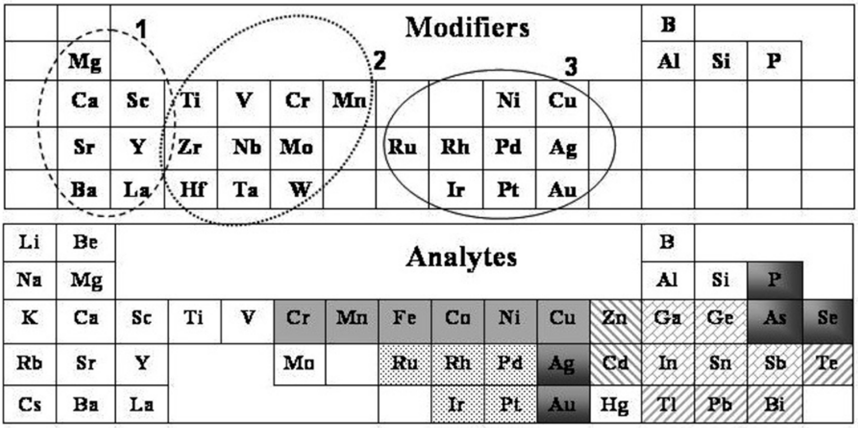

Figure 2. Classification of chemical modifiers (above) and volatile elements as analytes in ETAAS (below) (adapted from Tsalev and Slaveykova, 1992). 1: Group 1 modifiers – "Metals/Salt-like carbides"; 2: Group 2 modifiers – "Metals/Metal-like carbides"; 3: Group 3 modifiers – "(Noble) metals". Seven groups of volatile analytes given below. For details, see Tsalev and Slaveykova, 1992, 1998.

In spite of their large variety, chemical modifiers could be classified in several main groups (Figure 2), while numerous analytes with high and moderate volatility could be clustered into several groups with similarities in their behaviour in the graphite atomizer (Figure 2).

Over 50 chemical elements have been determined in biological and environmental materials, food and beverages as summarized in Table 6. For some of these analytes, the ETAAS is among the best available and documented analytical techniques in this field.

TABLE 6. Capabilities of ETAAS technique for determination of trace elements in biological, environmental and food matrices.

Sample matrix	*Trace element*
Air particulates (digests)	Ag, **Al**, **As**, Ba, **Be**, Bi*, **Cd**, Co, **Cr**, **Cu**, Fe, Ga, **Mn**, Mo*, **Ni**, **Pb**, **Sb**, **Se**, Sn*, Te*, Ti, V, Zn
Air particulates (SS)	Ag, As, Ba, Be, Bi*, **Cd,** Co, **Cr, Cu, Fe, Hg,** Li, **Mn, Ni, Pb,** Se, Si, Sn*, V, Zn
Air (after enrichment)[a]	As, Hg, Sb, Se, Te, Tl
Air (vapours)[a]	**As**, **Hg**, Mn, Ni, **Pb**, **Sb**, Se, Te
Blood (whole)	Al, As*, Au*, Bi*, **Cd**, Co*, Cr, **Cu**, Fe, Ga*, Hg, Mn, Mo*, Ni, P, **Pb**, **Pt***, Sb, Se, Si, Sn*, Te*, Tl*, V*, Zn
Blood plasma or serum	Al, As, Au*, Bi*, **Cd**, Co*, Cr, **Cu**, Fe, Ga*, Hg, Mn, Mo*, Ni, P, Pb*, **Pt***, Sb, **Se**, Si, Sn*, Te*, Tl*, V*, Zn
Fly ash (digests and leachates)	Ag, **Al,** As, **Be**, **Cd**, Co, **Cr**, **Cu**, Fe, **Ga**, **Ge**, **Hg**, **Mn, Ni, Pb,** Sb*, **Sc**, Se, Sn*, Ti, **Tl**, V, Zn
Fly ash (SS)	As, Cd, Cr, Cu, Ga, Ni, Pb, Se*, Tl, V
Food, feed, beverages (digests)	Ag*, **Al, As, Cd**, Co, **Cr, Cu, Fe, Hg**, **Mn, Ni**, **Pb**, Rb, Se, Si, **Sn**, Te*, V, Zn
Food, feed (SS)	Ag*, Al, As, **Cd**, **Cr**, Cs, **Cu**, **Fe**, **Hg**, **Mn**, Mo*, **Ni**, **Pb**, Rb, **Se**, Ti*, Tl*, **Zn**
Food, feed, beverages (after enrichment)[a]	**As**, Be, **Cd**, **Co**, Cr, **Cu**, **Hg**, **Mo, Ni, Pb**, Pt, Rh, **Sb, Se, Sn**, Tl, **V**
Hair or nail	Ag*, **As**, Au*, Ba, Bi*, **Cd**, Co, **Cr**, **Cu**, Fe, Hg, In*, **Mn**, Mo, Ni, **Pb**, Sb*, **Se**, Sn, Sr, Te*, Tl*, V, Zn
Milk (diluted, digests)	Al, As, Be*, **Cd**, Co*, **Cr**, **Cu**, Fe, Hg*, Li*, Ni*, Sb*, Se, Sn*, Tl*, V*, Zn
Milk (SS)	Cu, Fe, Zn
Sediments and sludges (digests, leachates)	**As**, Ba, Be*, Bi*, **Cd, Co, Cr, Cu**, Fe, **Hg**, Mn, Mo*, **Ni**, **Pb**, Sb, Se, **Sn**, Ti, Tl*, V
Sediments, sludges (SS)	Ag, **Al**, **As**, Bi*, Ca, **Cd, Co, Cr, Cu**, Fe, **Hg**, Mg, **Mn**, Mo*, **Ni**, **Pb**, Sb, Si, Sn*, Te*, Tl*, Zn
Soil (digests, leachates)	Ag, Al, **As**, Ba, Be*, **Cd, Co, Cr, Cu**, Hg*, Li, Mn, Mo*, **Ni**, **Pb**, Sb*, Se*, Sn, Sr, Ti, Tl*, V, Zn
Soil (SS)	Ag, **As, Cd,** Co, **Cr, Cu, Hg*, Ni, Pb**, Tl*
Tissues, marine (digests)	Ag*, Al, **As**, Ba, **Cd**, Co, Cr, Cs, **Cu**, Fe, **Hg**, **Mn**, Ni, **Pb**, Rb, **Se**, Sn*, Sr, Ti*, Zn
Tissues marine (slurried)	Al, **As**, **Cd**, Co, Cr, **Cu, Fe, Hg, Mn, Ni, Pb**, **Se**, V, **Zn**
Tissues, plant (digests)	Ag*, **Al**, B*, Ba, **Cd**, Co, **Cr**, Cs, **Cu**, Fe, Ge*, Hg*, Li, **Mn**, Mo*, **Ni, P, Pb**, Rb, Sb*, Se*, Sn*, Sr, Te*, Tl*, V*, Zn
Tissues, plant (slurried)	Ag*, **Al**, B, **Cd**, Co, **Cr**, **Cu, Fe,** **Hg*, Mn**, Ni, **Pb**, Rb, Ti*, Tl*, **Zn**
Tissues, soft (digests)	Ag*, **Al**, **As**, Au*, Be*, **Cd**, Co, Cr, **Cu**, **Fe**, **Hg**, Li*, **Mn**, Ni, P, **Pb**, Pt*, Rb, Ru*, Sb*, **Se**, **Si**, Sn*, Sr, Te*, Ti*, Tl*, V*, Zn

Tissues, soft (slurried)	Ag*, **Al**, **As**, **Cd**, Co, Cr, **Cu**, Fe, **Hg**, **Mn, Ni, Pb**, Pt*, Rb, Sb*, **Se**, Tl*, Zn
Water (high TDS)	Ag*, Al, As*, Ba, **Cd**, **Cr**, Cs, **Cu**, Li, **Mn**, Mo, Ni, **Pb**, Rb, Se*, Sr, V, **Zn**
Water (high TDS) after enrichment[a]	Ag, Al, As, Au, B, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ga, Ge, Hg, In, Li, Mn, Mo, Ni, Pb, Pd, Sb, Se, Sn, Te, Tl, V, Yb, Zn
Water (low TDS)	Ag, **Al, As**, B*, Ba, Be*, Bi*, **Cd, Co, Cr**, Cs, **Cu, Fe**, Hg*, Li, **Mn**, Mo*, **Ni**, P*, **Pb**, Rb, Sb*, Se*, **Si**, Sn*, Sr, Te*, Ti*, Tl*, V*, **Zn**
Water (low TDS) after enrichment[a]	Ag, Al, As, Au, Ba, Be, Bi, Cd, Co, Cr, Cu, Ga, Ge, Hg, In, Mn, Mo, Ni, Pb, Pt*, Rh*, Sb, Se, Sn, Te, Tl, V, Yb, Zn
Urine	Ag*, Al, **As***, **Au***, B*, Ba, **Be***, **Bi***, **Cd**, Co*, **Cu**, Fe, Ga*, Hg*, In*, Li*, **Mn**, Mo*, **Ni***, P, **Pb**, **Pt***, Rb, **Sb***, **Se***, Si, Sn*, Sr, Te*, **Tl***, V*, Zn

Adapted and updated from Tsalev, 1998, 2000. [a]Major enrichment step typically required at normal analyte concentrations; *Asterisk indicates applicability and good performance at abnormally high analyte levels, e.g. due to severe pollution and/or exposure; **bold** symbols indicate straightforward, competitive performance at normal analyte levels. SS, solid or slurry sampling; TDS, total dissolved solids.

2.4. VAPOUR GENERATION ATOMIC ABSORPTION SPECTROMETRY

VGAAS techniques are occupying an important niche in modern analytical determinations of ppb and sub-ppb levels of several trace elements and some of their molecular/redox species. They are readily available, more robust and less expensive as compared with some modern, more sensitive approaches such as AFS and ICP-MS. Technique requires a dedicated accessory, a vapour generator (hydride generator; mercury/hydride generator) where sample solution is mixed with a strong reductant (sodium tetrahydroborate(III) or sodium borohydride, $NaBH_4$). Other reductants can also be used bet are applied rarer – KBH_4 or $SnCl_2$ (the latter reagent for generation of Hg^o cold vapours only). Use of VG technique in a flow injection (FI) or continuous flow (CF) mode (Figure 3) offers certain advantages vs. batch type vapour generation (Dedina and Tsalev, 1995). The generated hydrides (AsH_3, SbH_3, H_2Se, etc.) are stripped out of solution by means of evolved H_2 and Ar purge gas and enter a heated quartz tube atomizer (QTA) or otherwise are treated in alternative ways: in situ trapping in a graphite atomizer (GA in Figure 3) with subsequent atomization in a VG–ETAAS (HG–ETAAS) system or (rarer) by employing other atomization modes (flame; 'flame-in-tube') (Karadjova et al., 2005, 2006) or even by another quantification technique (AFS, ICP-OES, ICPMS, etc.) (Dedina and Tsalev, 1995). Gross chemical reactions of hydrolysis of tetrahydroborate reagent BH_4^- and hydride generation can be given as: $BH_4^- + 3H_2O + H^+ \rightarrow H_3BO_3 + 4H_2\uparrow$ (with ca. 2,5 L of evolved H_2 per 1 g $NaBH_4$) and $H_3AsO_3 + 3BH_4^- + 3H^+ \rightarrow AsH_3\uparrow + 3BH_3 + 3H_2O$, although mechanisms of these reactions are stepwise and much more complicated (D'Ulivo, 2010). (*With caution:* vigorous reactions; highly toxic volatile products evolution; possible excessive foam formation!).

Some typical figures of merit for characteristic concentrations C_o in peak height measurements by CF-HGAAS (CF-CVAAS for mercury) and for HG–ETAAS with integrated absorbance A_{int} (peak area) measurements as well as typical instrumental LODs for these techniques are compiled in Table 7. Capabilities of VGAAS techniques for analyses of real samples are summarized in Table 8 (see relevant procedures in Dedina and Tsalev, 1995; Tsalev, 1995).

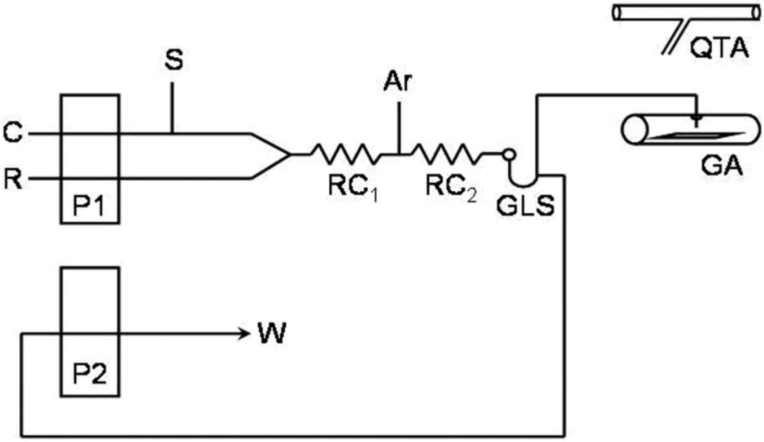

Figure 3. Scheme of AAS with hydride generation in flow injection (FI) mode with introduction of hydrides in a heated quartz tube atomizer (QTA), *viz.* FI-HGAAS (QTA) or in situ enrichment in a graphite atomizer (GA), *viz.* FI-HG–ETAAS. C, carrier; R, reductant flow; S, sample injection; P1 and P2, peristaltic pumps; RC_1 and RC_2, reaction coils; Ar, argon purge gas; GLS, gas-liquid separator; W, waste.

TABLE 7. Typical values for characteristic concentration (C_o), characteristic mass (m_o) and instrumental limit of detection (ILOD) for CF–HGAAS with QTA and HG–ETAAS techniques.

Analyte	*C_o, µg/L*	*m_o, pg*	*ILOD, pg*
As	0.1–0.2	11–43	10–24
Bi	0.04–0.2	10–34	>2–10
Cd	0.02–1	1–2.8	2.6–14
Ge	500	~10	
Hg[a]	0.03–0.2	>100	>100
In	>1,000		
Pb	0.3–0.8	~50	~10^3
Sb	0.08–0.3	13–14	>15
Se	0.1–0.3	~20	8
Sn	0.5–0.6	15–30	~70
Te	0.1–0.2	15–20	~10

[a] except for Hg by CVAAS or CV–ETAAS.

TABLE 8. Capabilities of VGAAS techniques (HGAAS, CVAAS, HG–ETAAS for determination of trace elements in biological, environmental and food matrices.

Sample matrix	*Trace element*
Air particulates (digests)	**As**, Bi*, Cd, Ge*, Pb, Sb*, Se*, Sn, Te
Air (vapours)	As, **Hg**, Pb, Sb, Se*, Sn*, Te*
Blood (whole)	As, Bi*, Cd, **Hg**, Pb, Sb, **Se**, Sn, Te
Blood plasma or serum	**As**, Bi*, Cd, **Hg**, Pb*, Sb, **Se**, Sn, Te*
Bone, tooth	As, **Hg**, Pb, Sb, Se
Fly ash	**As**, Bi, Cd, Ge, **Hg**, Pb, **Sb**, Se, Sn, Te, Tl*
Food (digests)	**As**, Bi*, Cd, **Hg**, Pb, Sb, **Se**, **Sn**
Food (slurries)	**Hg**, Pb
Hair or nail	**As**, Bi*, Cd, **Hg**, Pb, **Sb**, **Se**, Sn
Sediments and sludges	**As**, Bi, Cd, Ge, **Hg**, Pb, **Sb**, Se, **Sn**, Te
Soil	**As**, Bi, Cd, Ge*, **Hg**, Pb, **Sb**, Se, **Sn**, Te
Tissues, marine (digests)	**As**, Cd, **Hg**, Pb, Sb, **Se**, Sn
Tissues marine (slurried)	**Hg**, Pb
Tissues, plant (digests)	**As**, Bi, Cd, **Hg***, Pb, **Sb**, **Se**, Sn, Te
Tissues, plant (slurried)	Hg*, Pb*
Tissues, soft (digests)	**As**, Bi, Cd, **Hg**, Pb, Sb, **Se**, Sn, Te*
Tissues, soft (slurried)	**Hg**, Pb
Water (high TDS)	**As**, Bi, Cd, Ge*, **Hg** (with amalgamation), Pb, **Sb**, **Se***, Sn, Te
Water (low TDS)	**As**, Bi, Cd, Ge*, **Hg** (with amalgamation), Pb, **Sb**, **Se***, Sn, Te*
Urine	**As**, **Bi**, Cd, **Hg**, Pb, **Sb**, Se, **Sn**, **Te***

Adapted and updated from Tsalev, 2000; Dedina and Tsalev, 1995. *Asterisk indicates applicability and good performance at abnormally high analyte levels, e.g. due to severe pollution and/or exposure; **bold** symbols indicate straightforward, competitive performance at normal analyte levels. TDS, total dissolved solids.

2.5. COMBINED AND HYPHENATED VAPOUR GENERATION AAS TECHNIQUES

Flow techniques (CF, FI) offer many advantages in AAS (Fang, 1993, 1995; Sanz-Medel, 1999; Welz and Sperling, 1998) and particularly in VGAAS (review by Tsalev, 1999b). The following positive assets of flow injection (FI) techniques could be summarized: automation of sample introduction and on line manipulation (dilution, enrichment, derivatization, pre-reduction or pre-oxidation, removal of interferents, on-line pyrolysis, cryogenic trapping, etc.) in a closed, flow system by means of versatile autosamplers and FI manifolds; scaling down (sample volumes between 10 and 500 μL); good precision (a few percent RSD) and excellent short term repeatability; high sampling frequencies (e.g. 30–60 h^{-1}); lower absolute LODs; better interference control; possibility for on line treatments of liquid and (rarer) of slurried solid samples; (limited) capabilities for non-chromatographic speciation analysis, etc.

Liquid samples such as urine and environmental waters could be treated by on-line decomposition in a microwave oven (FI-MWD-HGAAS, FI-MWD-CVAAS) or UV-photodecomposition in a flow reactor (e.g. FI-UV-HGAAS for organoarsenicals, organotins), by on line pre-reduction in a flow reactor (FI-on-line pre-reduction/complexation with L-cysteine (Tsalev et al., 2000b), by on-line preconcentration and separation (see monographs by Fang, 1993, 1995 and Sanz-Medel, 1999), flow injection (FI) or continuous flow (CF) vapour generation with in-atomizer trapping, FI-HG–ETAAS, etc.

Continuous flow and flow injection systems exhibit potentialities for on-line speciation analysis by non-chromatographic and non chromatographic approaches. Among the former group are pH selective HG (Tsalev and Petrov, 1981), effects of oxidation state on HG (e.g. separation of Se(IV) from Se(VI)), derivatization of hydride forming species by complexation/pre-reduction with L-cysteine (Tsalev et al., 1996b, 2000b), use of pre-columns for solid phase extraction (SPE), etc. (reviews by Fang, 1993, 1995; Sanz-Medel, 1999; Welz and Sperling, 1998; Tsalev, 1995, 1999b; Gonzalvez et al., 2009, 2010). Numerous analytical schemes, instrumental systems and procedures for speciation analysis (HPLC-HGAAS, HPLC-UV-HGAAS and HG-GC-AAS) with chromatographic separations could be found in recent specialized monographs (Cornelis, 2005; Sanz-Medel, 1999).

Summary of some possible schemes for on-line treatments, enrichment and hyphenation in VGAAS are given in Table 9.

Some examples from the author's recent research in the field of FI-VGAAS applications could be presented here: (i) on-line decomposition of organoelement species in samples of urine and environmental waters in a flow injection system by means on-line UV photooxidation (UV) (Tsalev et al., 1998, 2000c) or microwave (MW) decomposition (Tsalev et al., 1992a,b); Welz et al., 1991); (ii) coupling of HPLC with UV-HGAAS (Tsalev et al., 1998, 2000c), (iii) in situ enrichment of HFEs in a HG–ETAAS system (Tsalev et al., 1995, 1996a,b; D'Ulivo et al., 1998; Tsalev et al., 2001; Lampugnani et al., 2003).

TABLE 9. Selected schemes vapour generation with on-line digestion, pretreatment, enrichment and determination in continuous flow or flow injection AAS systems.

		dign.	**VG**	**QTA**	
	dign	pre-redn.	VG	QTA	
		pre-redn.	VG	QTA	
			VG	**ETAAS**	
			VG	**amalgamation**	**QTA**
			VG	enrichment	QTA
		HPLC	**VG**	**QTA**	
	HPLC	**dign.**	**VG**	**QTA**	
HPLC	dign.	pre-redn.	VG	QTA	
			VG	**GC**	**QTA**
			VG	**CT/GC**	**QTA**

Most typical approaches are given in **bold**. Abbreviations: HPLC, high performance liquid chromatography, dign., on line digestion; pre-redn., on line pre-reduction; VG, vapour generation, GC, gas chromatography, CT, cryogenic trapping, QTA, atomization in quartz tube atomizer or flame-in-tube. For details, applications to As, Bi, Cd, Ge, Hg, Pb, Sb, Se, Sn, Te, etc. and references in the review by Tsalev, 1999b and monographs by Fang, 1993, 1995 and Sanz-Medel, 1999.

The automated on-line UV photolytic decomposition of some environmentally relevant organoarsenic and organotin compounds is useful with a view to both coupling HPLC with HGAAS detection (HPLC–UV–HGAAS) and species-independent quantification of the total arsenic or tin in samples containing various organic species (arsenite, arsenate, monomethylarsonate, dimethylarsinate, arsenobetaine, arsenocholine, Sn(IV), Me_2Sn, Me_3Sn, Et_3Sn, Pr_3Sn, Ph_3Sn, BuSn, Bu_2Sn, Bu_3Sn, Bu_4Sn) (Tsalev et al., 2000c); in some respect this approach is more robust than the on-line microwave decomposition (FI-MWD–CVAAS and FI-MWD–HGAAS) (Tsalev et al., 1992a, 1992b); Welz et al., 1991). Speciation analysis in flow-injection and hyphenated systems, e.g. HPLC–UV–HGAAS could thus be realized for the determination of As in urine (Tsalev et al., 1998, 2000c).

The on-line enrichment by VG with in-atomizer trapping of hydrides of As, Cd, Sb, Sn, Se, etc. in a heated graphite atomizer is efficient in the presence of permanent chemical modifiers (Zr–Ir or W–Ir). The integrated platform of the Transversely Heated Graphite Atomizer (THGA®) has been pre-treated with Zr (0.1–0.25 mg) or W (0.2–0.5 mg) and then with Ir (2–20 μg) for permanent modification as a basis for HG–ETAAS analytical procedures (Tsalev et al., 1995, 1996a,b; D'Ulivo et al., 1998; Lampugnani et al., 2003).

In a simpler, non-chromatographic approach for the determination of toxicologically relevant As in urine (the sum of As(III) + As(V) + MMA + DMA) have been proposed three procedures involving derivatization of these species to their corresponding L-cysteine complexes and subsequent HGAAS in batch, FI or FI-HG–ETAAS modes: (i) Batch HGAAS procedure, based on manual operation, with LOD 0.25 μg/L in 10 mL of 1 + 4 (v/v) diluted urine, integrated absorbance (peak area) measurements, with RSD 2–6% and sample throughput rates 15 h^{-1} (Georgieva et al., 2007); (ii) FI-HGAAS procedure (automated), with 500 μL injections of 10 fold diluted urine, with LOD of 1 μg/L, RSD 2% and sample throughput rates of 50 h^{-1} (Guo et al., 1997); and a FI-HG–ETAAS procedure (automated), based on in-situ trapping of hydrides from 500 μL of 10–25 fold diluted urine, with LOD of 0.4–1 μg/L, respectively, RSD ~ 1% and sample throughput rates of 25 h^{-1} (Petrov et al., 2006).

2.6. RECOMMENDED EXPOSURE TESTS BASED ON AAS MEASUREMENTS

On the basis of the author's experience with the AAS techniques applied to various chemical elements in environmentally and health related materials (Table 10) and ample literature data from this vast area (Tsalev, 1995; Tsalev and Zaprianov, 1983) some relevant combinations of element–sample–recommended technique could be summarized (Table 11). For most of these tests, the AAS technique is the routine current method-of choice for the determination of one or a few elements per sample. For large sample throughput, multielement analyses, however, the modern methodologies of ICP-QMS with in situ treatment of interferences by collision/reaction cells (in biological and food area as well as in water analysis) and ICP-OES techniques (in environmental and agricultural field of applications) are nowadays broadly introduced, extensively developed and researched, in spite of their higher investment and running costs.

TABLE 10. Analytes and matrices studied by the author (see also Tsalev, 1995).

Blood (Al, Cd, Cr, Mn, Pb, Se), *bone* (Pb), *erythrocytes* (Cr), *essential oils* (As), *fish and marine tissues* (As, Cu, Pb, Se), *hair* (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, Zn), *nail* (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, Zn), *pharmaceuticals* (As, Cd, Co, Cu, Ni, Fe, Pb, Zn), *plant tissues* (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn), *seawater* (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Sb, Se, Zn), *sediment* (As, Pb, Sb, Se, Sn, Tl), *serum* (Co, Cu, Fe, Mn, Ni, Pb, Se, Zn), *soil* (As, Bi, Cd, Pb, Sb), *soil extracts* (As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn), *tissues and organs* (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, Zn), *urine* (As, Bi, Cd, Cr, Hg, Mn, Pb, Sb, Se, Sn, Tl), *water* (As, Bi, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, Sn, Zn), *wine* (As, Pb), and *misc. materials* (As, Bi, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sn, V, Zn).

TABLE 11. Some possible toxicokinetic tests for biological monitoring given as a combination of element–sample–recommended technique (see details on procedures in Tsalev, 1984, 1995 and recent reviews).

Ag-B-ETAAS, Ag-U-ETAAS
Al-S-ETAAS, Al-H-ETAAS, Al-U-ETAAS
As-U-HGAAS, As-U-ETAAS, As-N-ETAAS, As-H-ETAAS
Au*-B-ETAAS, Au*-P/S-ETAAS, Au*-U-ETAAS
Be-U-ETAAS, Be-P/S-ETAAS
Bi*-U-HGAAS, Bi*-U-ETAAS
Cd-B-ETAAS, Cd-U-ETAAS, Cd-H-ETAAS
Co-B-ETAAS, Co-S/P-ETAAS, Co-U-ETAAS
Cr-S/P-ETAAS, Cr-U-ETAAS, Cr-H/N-ETAAS, Cr-Er-ETAAS
Cu-S-FAAS, Cu-U-ETAAS
Fe-S-photom., Fe-S-FAAS
Hg-B-CVAAS/CVAFS, Hg-U-CVAAS/CVAFS, Hg-H-CVAAS
Li*-P/S-FAES/FAAS, Li*-U-FAES/FAAS, Li*-saliva-FAES/FAAS, Li*-B-FAAS
Mn-U-ETAAS, Mn-H/N-ETAAS
Ni-U-ETAAS, Ni-P/S-ETAAS, Ni-B-ETAAS
Pb-B-ETAAS, Pb-U-ETAAS, Pb-H/N-ETAAS
Pt*-U-ETAAS, Pt*-P-ETAAS
Se-P/S-ETAAS, Se-U-ETAAS/HGAAS, Se-B-ETAAS
Tl*-U-ETAAS, Tl*-B-ETAAS
Sn-U-HGAAS
Zn-P/S-FAAS, Zn-U-FAAS

*Asterisk denotes assays at therapeutic or acute toxicity levels. Abbreviations: B, blood; CVAAS, cold vapour atomic absorption spectrometry; CVAFS, cold vapour atomic fluorescence spectrometry; Er, erythrocytes; FAAS, flame atomic absorption spectrometry; FAES, flame emission photometry; HGAAS, hydride generation atomic absorption spectrometry; photom., molecular absorption spectrophotometry; P/S, blood plasma or serum; H, hair; N, nail; U, urine.

3. Quality Control and Quality Assurance Considerations in Trace Element Analyses

Metrological considerations for traceability of analytical results and evaluation of their uncertainty play an indispensable role in modern trace element analysis. Quality control (QC) and quality assurance (QA) practices are well treated in recent metrological literature (Hibbert, 2007) and ISO Guides. The most important metrological considerations and methodological approaches used in method development, validation, verification and QC/QA are as follows: the use of appropriate blanks and traceable chemical calibrants; control of sensitivity drift and baseline drift; replication of measurements (parallels; duplicate samples, incl. 'blank duplicates'); analyses of relevant matrix Certified Reference Materials (CRM); analysis of spiked samples (spike recovery R% evaluation); evaluation of robustness/ruggedness of measurement procedures; comparison of results of evaluated procedure with those obtained by an accepted procedure; comparison of results by using calibration curve vs. standard additions calibration; routine use of various control charts; regular participation in interlaboratory comparison exercises and in proficiency testing (PT) (external quality assessments), etc. The importance of RMs and CRMs for traceability of chemical measurements cannot be exaggerated.

In Table 12 are summarized data about availability of CRMs for elemental contents in trace elements in biological, environmental, food and related materials. More information on current RMs, CRMs, standard solutions, and quality control materials could be assessed via web sites of numerous producers and distributors: COMAR, the International Database for CRMs <www.comar.bam.de> with over 10,500 CRMs from over 200 producers from more than 20 countries; the International Atomic Energy Agency (IAEA, Vienna) <www.iaea.org> "The IAEA Database of Natural Matrix Reference Materials" <www-naweb.iaea.org/nahu/external/e4/nmrm>; the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) <www.irmm.jrc.be> and <www.irmm.jrc.cec.eu.int>, a major producer of CRMs, isotopic CRMs and ERM® (European Reference Materials <www.erm-crm.com> in collaboration with LGC Standards in UK <www.lgcstandards.com> and и BAM in Germany <www.bam.de>; the National Institute of Standards and Technology (NIST, USA <www.nist.gov>) with over 1,300 SRM® (Standard Reference Materials and NTRM® (NIST Traceable Reference Materials); the Virtual Institute for Reference Materials (VIRM) <www.VIRM.net> with over 24,500 records analyte/matrix and ca. 4500 RMs and CRMs; the Institute for National Measurement Standards (Ottawa, Canada); the National Institute for Environmental Studies (Tsukuba, Japan), etc. RMs and CRMs for speciation analysis are scarce, covering partly leachable or extractable fractions in dust, soil, sediment as well as a limited number of chemical elements and relevant species, e.g. As, Cr, Hg, Pb, Se, Sn (Table 12).

TABLE 12. Availability of certified reference materials for elemental contents in biological, environmental, food and related materials (matrices and analytes in alphabetical order).

Air particulate matter, dust, vehicle exhaust particulates: Al, As, Ca, Cd, Co, Cr (total), Cr(VI), Cr (total leachable),Cu, K, Mg, Na, Ni, Pb (total), $PbMe_3$, Pd, Pt, Rh, Sb, Sr, V, Zn
Ash (fly ash, incinerator ash, etc.): As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Na, Ni, Pb, V, Zn
Blood (whole): As, Cd, Cr, Cu, Fe, Hg (total, inorganic, CH_3Hg), Ni, Pb, Se, Tl, Zn
Blood plasma or serum: As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mo, Ni, Pb, Se, Zn
Bone: Ba, Fe, Pb, Sr, Zn
Coal: Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, K, Mn, Na, Ni, Pb, Rb, Sc, Se, Ti, Tl, V, Zn
Food: Al, As, Au, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Zn
Hair: Ba, Cd, Cr, Cu, Fe, Hg (total, CH_3Hg), Mn, Pb, Sb, Se, Zn
Milk: Al, As, Au, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, P, Pb, Sb, Se, Se, Tl, Zn
Paint (leachable contents): As, Ba, Cd, Cr, Pb, Sb, Se
Polyethylene: As, Cd, Cr, Hg, Pb
Sediment: Al, As, Be, Cd, Co, Cr, Cu, Fe, Hg (total, CH_3Hg), Mn, Mo, Ni, Pb, Pd, Pt, REE, Sb, Sc, Se, Sn (total, C_4H_9Sn, $(C_4H_9)_2Sn$, $(C_4H_9)_3Sn$, C_6H_5Sn, $(C_6H_5)_2Sn$), Sr, Ti, U, V, Zn
Sewage sludge: Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Zn
Soil: As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, REE, Sb, Sc, Se, Sn, U, Zn
Soil and sludge (extractable contents): Cd, Cr, Cu, Ni, Pb, Zn
Tissues (animal): Al, As, Au, Ca, Cd, Co, Cr, Cu, Fe, Hg (total, CH_3Hg), K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sr, V, Zn
Tissues (aquatic): Ag, Al, As (total, AsBet, $(CH_3)_2As$), Au, Ca, Cd, Co, Cr, Cu, Fe, Hg (total, CH_3Hg), K, Mg, Mn, Mo, Ni, Pb, REE, Sb, Se, Sn (total, C_4H_9Sn, $(C_4H_9)_2Sn$, $(C_4H_9)_3Sn$), Sr, U, V, Zn
Tissues (terrestrial plants): Al, As, Au, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, P, Pb, Rb, REE, Sb, Se, Si, Sr, Tl, U, Zn
Urine: Al, As (total, AsBet, $(CH_3)_2As$, Be, Ca, Cd, Co, Cr, Hg, Mg, Mn, Na, Ni, Pb, Sb, Se, Tl, Zn
Water (low TDS): Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sr, U, V, Zn
Water (sea, estuarine): Al, As, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se(IV), Se (total), U, V, Zn

Abbreviations: AsBet, arsenobetaine; REE, rare earth elements (lanthanoids); TDS, total dissolved solids. See also respective sites and catalogues as given in text above.

4. Compilation of Some Reference Values for Bulgaria

A compilation of published values on trace element concentrations in biological and environmental samples for non-exposed sites and individuals from Bulgaria, based mainly on AAS techniques from author's own research is given in Table 13 for reference purposes.

TABLE 13. Compilation of some reference values for biological and environmental materials from Bulgaria (from non-exposed individuals and non-polluted sites, resp.), arranged in alphabetical order by elements and matrices.

Aluminium

Nails (toe): Mean 27.4 ± 32.8 (SD) μg/g, range 1.1–122, median 15.1 μg/g (n = 24 males) (Tsalev et al., 1993).

Antimony

Nails (toe): Mean 0.35 ± 0.17 (SD) μg/g, range 0.021–0.70, median 0.31 μg/g (n = 20, males) (Tsalev et al., 1993).

Arsenic

Nails (toe): Mean 0.58 ± 0.44 (SD) μg/g, range 0.13–1.60, median 0.36 μg/g (n = 18, males) (Tsalev et al., 1993).

Soil: 2.5–11.5 μg/g; various types non-polluted soils (Petrov and Tsalev, 1979); 2.0–11.2 μg/g various types non-polluted soils (Petrov and Tsalev, 1979; Tsalev and Petrov, 1981).

Urine (toxicologically-relevant fraction of As(III) + As(V) + MMA + DMA): controls mean 3.5 ± 2.1 (SD), range 0.9–10.4, median 3.0 μg/L (n = 99); a group of individuals whose father or mother were included in the Vratza hospital registry of Balkan Endemic Nephropathy (BEN) patients (n = 102), mean 3.6 ± 2.2 (SD), range 0.5–11.0, median 3.2 μg/L (no statistical difference from control group). On the basis of smoking habits: non-smokers (n = 127), mean 3.3 ± 2.1 (SD), range 0.6–11.0, median 2.8, interquartile range (25–75%) 1.8–4.3 μg/L and smokers (n = 74), mean 3.9 ± 2.1 (SD), range 0.5–8.8, median 3.6, interquartile range (25–75%) 2.4–5.0 μg/L (no statistical difference between these two groups: F-test (p = 0.75), t-test (p = 0.07) (Georgieva et al., 2007).

Cadmium

Nails (toe): Mean 0.13 ± 0.17 (SD) μg/g, range 0.01–0.91, median 0.08 μg/g (n = 29, 19 males, 10 females); mean 0.13 ± 0.20 (SD) μg/g, range 0.01–0.91, median 0.08 μg/g (n = 19, males); mean 0.12 ± 0.12 (SD) μg/g, range 0.02–0.39, median 0.08 μg/g (n = 10, females) (Tsalev et al., 1993).

Soil: 0.24–0.76 μg/g for various types of non-polluted soils (Petrov and Tsalev, 1979); 0.11–0.76 μg/g various types of non-polluted (Petrov and Tsalev, 1982).

Seawater: Range 0.05–0.16 nM/L (Black Sea coastal water, 7 locations; total dissolved metal concentration following 0.22 μm filtration) (Slaveykova et al., 2009).

Chromium

Nails (toe): Mean 1.3 ± 1.3 (SD) μg/g, range 0.3–7.6, median 0.9 μg/g (n = 42, 21 males, 21 females); mean 1.0 ± 0.6 (SD) μg/g, range 0.3–2.1, median 0.8 μg/g (n = 21, male); mean 1.6 ± 1.7 (SD) μg/g, range 0.4–7.6, median 1.0 μg/g (n = 21, females) (Tsalev et al., 1993).

Urine: 0.1–1.5 μg/L (Zaprianov et al., 1986).

Cobalt

Nails (toe): Mean 0.027 ± 0.012 (SD) μg/g, range 0.013–0.053, median 0.026 μg/g (n = 21, 15 males, 6 females); mean 0.024 ± 0.010 (SD) μg/g, range 0.013–0.051, median 0.021 μg/g (n = 15, males) (Tsalev et al., 1993).

Copper

Nails (toe): Mean 2.6 ± 1.4 (SD) µg/g, range 0.1–6.0, median 2.4 µg/g (n = 18, 7 males, 11 females); mean 3.0 ± 1.4 (SD) µg/g, range 1.8–5.7, median 2.4 µg/g (n = 7, males); mean 2.5 ± 1.5 (SD) µg/g, range 0.1–6.0, median 2.4 µg/g (n = 11, females) (Tsalev et al., 1993).

Seawater: Range 5.8–18 nM/L (Black Sea coastal water, 7 locations; total dissolved metal concentration following 0.22 µm filtration) (Slaveykova et al., 2009).

Iron

Nails (toe): Mean 13.4 ± 10.1 (SD) µg/g, range 4.2–28.7, median 10.8 µg/g (n = 41, 22 males, 19 females); mean 12.6 ± 6.6 (SD) µg/g, range 4.2–31.5, median 13.6 µg/g (n = 22, males); mean 14.2 ± 13.2 (SD) µg/g, range 4.2–48.7, median 9.9 µg/g (n = 19, females) (Tsalev et al., 1993).

Lead

Nails (toe): Mean 1.7 ± 1.9 (SD) µg/g, range 0.1–8.8, median 1.0 µg/g (n = 40, 19 males, 21 females); mean 1.1 ± 0.9 (SD) µg/g, range 0.2–2.3, median 0.9 µg/g (n = 19, males); mean 2.3 ± 2.3 (SD) µg/g, range 0.1–8.8, median 1.6 µg/g (n = 21, females) (Tsalev et al., 1993).

Seawater: Range 0.12–0.21 nM/L (Black Sea coastal water, 7 locations; total dissolved metal concentration following 0.22 µm filtration) (Slaveykova et al., 2009).

Manganese

Nails (toe): Mean 0.8 ± 0.5 (SD) µg/g, range 0.1–2.7, median 0.6 µg/g (n = 49, 26 males, 23 females); mean 0.9 ± 0.5 (SD) µg/g, range 0.2–2.7, median 0.8 µg/g (n = 26, males); mean 0.6 ± 0.4 (SD) µg/g, range 0.1–1.7, median 0.5 µg/g (n = 23, females) (Tsalev et al., 1993).

Nails (toe): geometric mean ± geom. SD 0.8 ± 1.3 µg/g (n = 9; 6 males, 3 females; urban, non exposed) (Zaprianov et al., 1985).

Nails (toe): range 0.1–2.7 µg/g; geom. mean ± geom. SD 0.5 ± 1.1 µg/g (n = 23 males); 0.8 ± 1.1 µg/g (n = 26 females); urban, non exposed from four towns: (A) 0.8 ± 1.3 µg/g (n = 9), (B) 0.6 ± 1.2 µg/g (n = 17), (C) 0.8 ± 1.1 µg/g (n =16), 0.4 ± 1.3 µg/g (n = 7) (Zaprianov et al., 1988, 1989).

Urine: 1.4 ± 0.4 µg/6 h (n = 14) (urban, non exposed) (Zaprianov et al., 1989).

Molybdenum

Nails (toe): Mean 0.061 ± 0.032 (SD) µg/g, range 0.017–0.127, median 0.062 µg/g (n = 20) (Tsalev et al., 1993).

Nickel

Nails (toe): Mean 0.23 ± 0.22 (SD) µg/g, range 0.04–1.04, median 0.14 µg/g (n = 30, 22 males, 8 females); mean 0.20 ± 0.16 (SD) µg/g, range 0.04–0.49, median 0.16 µg/g (n = 22, male) (Tsalev et al., 1993).

Seawater: Range 7.9–8.9 nM/L (Black Sea coastal water, 7 locations; total dissolved metal concentration following 0.22 µm filtration) (Slaveykova et al., 2009).

Selenium

Blood serum: 57 ± 14 µg/L in blood serum (n = 345) (Tzatchev, 1994).

Blood serum: for 24 paired samples of blood serum and urine from non-exposed individuals in Sofia, BG, serum selenium 66.5 ± 15.5 (45.5–116.4 µg/L (n = 24, 10 males and 14 females, age 18–60 years). No significant difference revealed between women 66.2 ± 18.0 (45.5–116.4 and men 66.9 ± 12.3 (47.2–84.9) µg/L in serum.

Nails (toe): Mean 0.34 ± 0.18 (SD) µg/g, range 0.05–0.61, median 0.34 µg/g (n = 16 (Tsalev et al., 1993).

Urine: for 24 paired samples of blood serum and urine from non-exposed individuals in Sofia, BG, urine selenium 16.8 ± 8.1 (6.0–41.9) µg/L (n = 24, 10 males and 14 females, age 18–60 years) (men). No significant difference revealed between women 17.8 ± 8.8 (8.5–41.9) µg/L and men 15.4 ± 7.2 (range 6.0–27.9) µg/L in urine (Tsalev et al., 2001).

Zinc

Nails (toe): Mean 87.7 ± 21 (SD) µg/g, range 50.3–149, median 86.0 µg/g (n = 24, 9 males, 15 females); mean 83.3 ± 20.2 (SD) µg/g, range 50.3–129.6, median 83.1 µg/g (n = 15, females) (Tsalev et al., 1993).

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5. Conclusions

Generalizations may well prove risky and even misleading in a huge area such as trace element research and laboratory practice, where are operating thousands of laboratories, performing many millions of measurements each year. Up to fifty chemical elements are determined at low concentrations (within 10^{-1}–10^{-10}%) in hundreds of complex matrices with countless number of individually varying sample compositions. Several major analytical techniques with high sensitivity and selectivity are successfully operating in this field, being rather supplementary than rivalling to each other: AAS with its five main options (FAAS, ETAAS, CVAAS, HGAAS, and hyphenated AAS techniques), ICP-OES, ICPMS, and VGAFS.

An attempt was made in this overview to point out the analytical scope, metrological and technical characteristics, major problems, general sample preparation rules, applicability of each AAS technique to real biological, environmental, food and related sample matrices, as well as to give some examples and to outline some trends on the basis of the author's own research and experience during the last several decades.

Selection of an appropriate instrumental method and particular analytical procedure is governed by various considerations with metrological, technical, methodological, and economic character. The mission of individual laboratory and the scope of its tasks such as routine monitoring, versatility of research topics, contract laboratory with high sample load, single element tasks (e.g. only Pb in blood or Hg in marine food) or simultaneous determination of many elements in each sample – all these individual considerations are overweighing and decisive in individual cases.

AAS techniques have reached a state of maturity and are ranking very high in many thousands of laboratories for trace element analysis: they are widespread at affordable price and with low running costs; high degree of automation of instrumental measurements has been reached (except for some batch type VGAAS instruments); many procedures are standardized and have been tested in routine practice with good degree of validation. FAAS (for up to 20 elements) and CVAAS techniques (for Hg) are robust, reliable and tolerable to high TDS. ETAAS (for up to 30 elements) provides very low LODs in the pg range, relies on microsampling with slurries, solids and simply pretreated or intact biological fluids, with in-situ ashing of the organic matter. HGAAS provides good relative sensitivity and low LODs (0.1–1 μg/L) in solutions, digests or leachates for several difficult analytes (As, Sb, Se, Sn, etc.), with attractive sampling frequencies (20–60 h^{-1}); it also exhibits speciation capabilities. HG–ETAAS provides LODs down to 0.005–0.01 μg/L) but is more expensive than HGAAS and ETAAS. CVAAS technique with amalgamation is among the best current techniques for Hg, with LODs down to a few ng/L. Dedicated CVAAS and CVAFS photometers are available as trace mercury analyzers.

VGAFS is a more sensitive alternative to VGAAS (better LODs by ca. one order of magnitude) and is accordingly more suitable as chromatographic detector. The powerful ICP-OES and ICPMS techniques are competitive in simultaneous or very fast sequential mode (for more than 4–5 elements per sample) in high sample throughput laboratories; they cover larger number of elements and impressively broader dynamic range of analyte concentrations. The ICPMS method is also attractive for its unique sensitivity and very low LODs, as well as a chromatographic detector (HPLC-ICPMS).

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IN SITU ELEMENTAL AND ISOTOPIC ANALYSES OF HEAVY METALS IN ENVIRONMENTAL WATER SOLUTIONS

BIANA SIMEONOVA[1*], LUBOMIR SIMEONOV[2]
AND CHRISTOPH SCHMITT[3]
[1] *Institute of Electronics*
Bulgarian Academy of Sciences
72 Tzarigradsko Shossee Bul, 1784 Sofia, Bulgaria
[2] *Solar-Terrestrial Influences Institute*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str. Bl.3, 1113 Sofia, Bulgaria
[3] *Dr. Schmitt-ISOTOP*
Laboratory for Laser Analytical Research
Sauerwiesen 6, 67661 Kaiserslautern-Siegelbach, Germany

Abstract. Presented are results of applicability tests of a miniature and portable time-of-flight laser mass-analyzer with water solutions. Discussed are the analytical possibilities of the laser time-of-flight mass spectrometry to produce express and stable elemental and isotopic mass spectra of environmental aqueous samples directly *in-situ* of the places of sample collection and several related problems as sample preparation, multielement chemical analysis and reliability of analytical results. A description of a simple methodic for preparation of laser targets from water solutions with different concentrations of the metals Na, Mg, Fe, Ni, Cd and Hg is presented. In a descending row from 1,000 to 62.5 ppb and in quantities of a 0.25 ml each, the solutions were carefully evaporated on glass holders and the produced sedimentation residues were investigated with the laser mass analyzer. The data presented below showed a deviation within 16% from the expected values, which is a proof of quite satisfactory sample homogeneity. The possible areas and cases of the proposed methodic for express target preparation are also discussed.

Keywords: Heavy metals, elemental and isotopic analysis, laser mass spectrometry, environmental chemical analysis, *in-situ* analysis

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[*] To whom correspondence should be addressed: bianasimeonova@abv.bg

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1. Introduction

The determination of the elemental and isotopic composition of solid samples can be achieved by the implementation of different analytical instrumentation and methods. A great percentage of the samples of interest to be analyzed are in a liquid state, as in the case of periodic tests of drinking water composition; in cases of local contamination of partially individual water volumes after an industrial accidents or acts of criminal pollution; in laboratory tests in medical practice or in criminology. In such occasions a relatively fast and inexpensive *in-situ* analysis is often needed. The standard methodic like AAS (Atomic Absorption Spectrometry) and ICP (Inductively Coupled Plasma) spectrometry suffer from several restrictions, which make them inappropriate for the purpose. The disadvantages of AAS are that only one element can be determined at a time and the dynamic range is relatively small. Some elements, as Ti, Mo and Si cannot be detected with AAS even at high concentrations. In the ICP spectrometry the use of spectral reagents, solvents, transport gases and the entire sample preparation process inserts risks of introduction contaminants and loss of elements. Disadvantage is also the high consumption of electrical energy and carrier gases, what limits severely the number of potential places of application of these methodic.

The choice of implementation of laser mass spectrometry in water solutions analysis is explained by several main outlines: the possibility to obtain analytical information about the whole m/q range from a single laser shot/mass spectrum, i.e. to achieve a multielement analysis performance; semi-quantative capabilities as a minimum; relatively high sensitivity; big dynamic range and sufficient mass resolution (Moenke-Blankenburg, 1989; Schmitt, 1994). These characteristics, in combination with the specific technical parameters of the portable laser mass analyzer (the instrument requires no special room and the installation could be accomplishes on a small desk; the small dimensions of the device, the simple operation procedure and the automatic data processing allow its transportation by any vehicle and a functioning in field conditions at power consumption below 1 kW; etc.), makes its implementation in the express water solution analysis quite appreciable.

In principle, liquid samples could be examined by laser mass analyzers, if they are transformed into a solid aggregate state. This is necessary because the sample holders are normally in high vacuum. The usual procedure to achieve a solid state of the aqueous samples is to freeze them in a specially designed sample holder system by the help of a liquid nitrogen cooling. This method unfortunately has disadvantages, which limit its scopes of application and does not give the expected results. The disadvantages are in connection with several main reasons: the sample is easily destroyed and needs additional time for cooling before the next laser shot; the vacuum in the sample holder department is deteriorated and needs to be restored; the vacuum system could easily be contaminated; in case of water solutions the small abundances, which constitute the main analysis interest could easily be lost in the bulk of water; the related problems of finding, preservation and handling of the liquid nitrogen.

An alternative and very promising method for the preparation of solid samples and laser targets from water solutions is presented below, featuring temperature-controlled evaporation of the water constituent and mass analysis of the solid residue with the laser mass analyzer.

2. Experimental Setup

The experiments were performed with the miniature laser time-of-flight analyzer LASMA (**LAS**er **M**ass **A**nalyzer). The initial scientific idea of the LASMA instrument belongs to Prof. Georgy Managadse from the Space Research Institute in Moskow. The first developments of the device were built for the purposes of space and planetary research and especially for investigation of the regolith of small bodies of the Solar System from the board of the lander. The environmental applications of the instrument are result of a technological transfer with preservation of the main concept and the functional building units. The earth-based instrumental developments preserve also the main features of a space instrumentation as small dimensions, reliability, easy operation, control and management, and stability of the analyzing parameters. The instrument consists of the following main functional units: the vacuum chamber with pumping-out, providing a working vacuum of 5 x 10^{-6} mbar or lower; a vacuum lock with a movable sample holder and a separate pumping outlet, which makes it possible for a sample replacement to be achieved within 1 min without any gas leakage in the main vacuum volume of the instrument; the laser itself is a Q-switched NdYAG at 1,064 nm; a beam focusing system; an electrostatic reflector; a micro-channel chevron particle detector of a non-standard configuration; a power supply unit; the automatic data acquisition system includes a transient recorder with a sampling rate of 100 Mhz (LeCroy model 9400A) a laptop and a printer (Schmitt, 1994; Schmitt and Schmiedel, 2007; Simeonov et al., 2005; Wohl et al., 1999). The point of irradiation on the surface of the sample is observed with the help of a microscope and can be easily changed and adjusted with the movable micrometer sample holder with respect to the laser-beam focus. The laser mass analyzer LASMA has the following analytical characteristics: mass range from 1 to 250 a.m.u.; mass resolution (FWHM) not lower than 200; maximum resolution in individual spectra of a series up to 600 and relative sensitivity of one analysis 10^{-5}–10^{-6}.

3. Sample Preparation Setup

The evaporation setup was used in experiments with a target to investigate the possibilities to measure fluid (water) samples with the laser mass spectrometer without preliminary chemical preparation of the samples. On Figure 1 is presented a schematic drawing of the temperature-controlled evaporation unit for the preparation of residue laser targets from metal containing water solutions. The unit consists of an electric resistance heater, mounted on an isolated with Teflon aluminum frame, a NTC temperature couple sensor and a standard 30 V/1.5 A power supply.

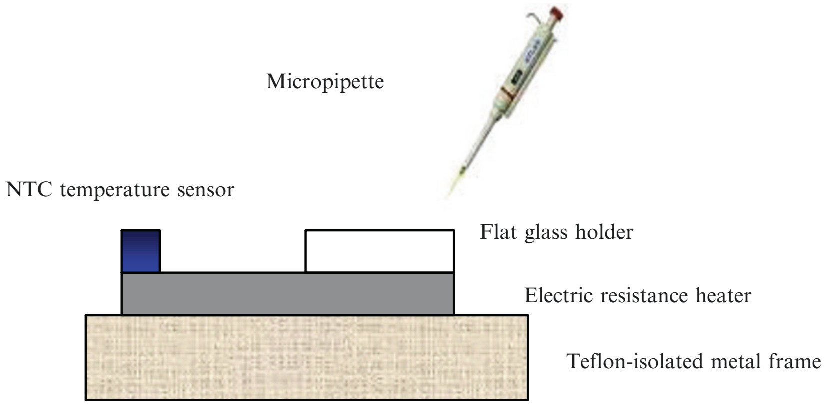

Figure 1. Schematic drawing of the temperature-controlled evaporation unit for laser target preparation out of water solutions.

Two types of sample holders were used, a 0.5 mm thick glass flats and Teflon minicups, with a volume to contain 1.05 ml of liquid. The water solution for evaporation was given in small drops with the help of a standard micropipette or by a 1 ml syringe with a special cut of the needle tip, in order to achieve an accurate, uniform and precise delivery of the drops. The liquid solutions were prepared by adding well known amounts of chemical elements from atomic standard solutions to a normal drinking water (in order to work in a real matrix), producing a 1,000 ppb concentrations for each element in the first order. By thinning with water, further concentrations of 500, 250, 125 and 62.5 ppb were provided. The elements added were Na, Mg, Fe, Ni, Cd and Hg. In order to have a stable reference for every solution, the concentration of Ni was kept constant at 2 ppm. The atomic standard solutions, used in the experiment, were from Aldrich and Baker Analyzed, and are prepared by dissolving metals of high spectral purity in HNO_3 and HCl acids and diluting with deionizer water. The sample preparation itself was achieved by a careful delivery of tiny drops of the solution on the preliminary cleaned with acetone surface of the glass flat. The glass flat, which is placed on top of the heater is maintained at constant temperature not exceeding 60°C, which was strictly tracked in order to escape from losses of the low temperature volatile elements (e.g., Cd or Hg), which was observed at several preliminary experiments. The so defined heating conditions allow the evaporation of an equal quantity of 0.25 ml from every concentration to be accomplished within 20 min. The evaporation process could be observed and controlled by a microscope. The sedimentation residue of every consequent drop of the solution forms a thin film with a very well defined circle shape and distinct boarder, which keeps the next drop and its residue within the circle. Every following drop, when evaporated, adds an approximately equal quantity of sedimentation material to the film and on one and a same spot. The preliminary evaluation of the evaporation process showed that a total quantity of 0.25 ml fills uniformly the spot area and is enough for a sufficient sedimentation. The glass flat with so prepared target (every concentration on a separate glass flat), marked beforehand, is mounted on the movable sample holder and inserted in the vacuum lock of the laser mass analyzer. The sample holder unit is pumped out for approximately 1 min and the instrument is ready to take-off the first mass spectra of the new sample.

4. Results and Discussions

Twenty five mass spectra from each sample (corresponding to each solution from 1,000 ppb to 62.5 ppb) with good mass resolution were recorded and taken in consideration for a sufficient statistics. The procedure was the following: The time-of-flight spectra are preliminary evaluated on a transient recorder, whose parameters were set to allow a complete view of the spectrum range between 0 and 5,000 nsec. The time-of-flight data is stored in the memory of the personal computer for a further processing. This is done with the help of a computer program for recording, analysis and interpretation of the mass spectra, especially designed for performance of the LASMA device data. The data analysis is done in several consecutive steps. First of all, the time-of-flight spectra are converted into mass spectra, i.e. the measured time-of-flights are expressed in exact mass numbers through a mass-calibration procedure. In this mode of operation the small time-of-flight fluctuations from one laser shot to another can be taken into consideration. The precision mass peak identification is achieved through the examination of the low-intensity mass peak structures. The second step of data evaluation is the calculation of the mass peak intensities and the peak area, in accordance to the isotope positions in the mass scale and the natural abundances ratios between the isotopes of every single element, which is presented in the spectra. For every measured mass peak the program works out a peak-fitting procedure in up to five steps of consequent approximation as the number of fitting steps depend on the complexity of mass-peak identification. After the peak-fit is completed, the program stores a mass spectrum and gives a printout of the most appropriate fit. On Figure 2 is given a part of a mass spectrum in the region of Cd after peak-fit identification. The so-called fingerprint of Cd presents every of the natural isotopes of the element. In this way an information about the relative (in percentage) and absolute (the peak-area in arbitrary units) abundance of every identified element in the spectrum could be obtained.

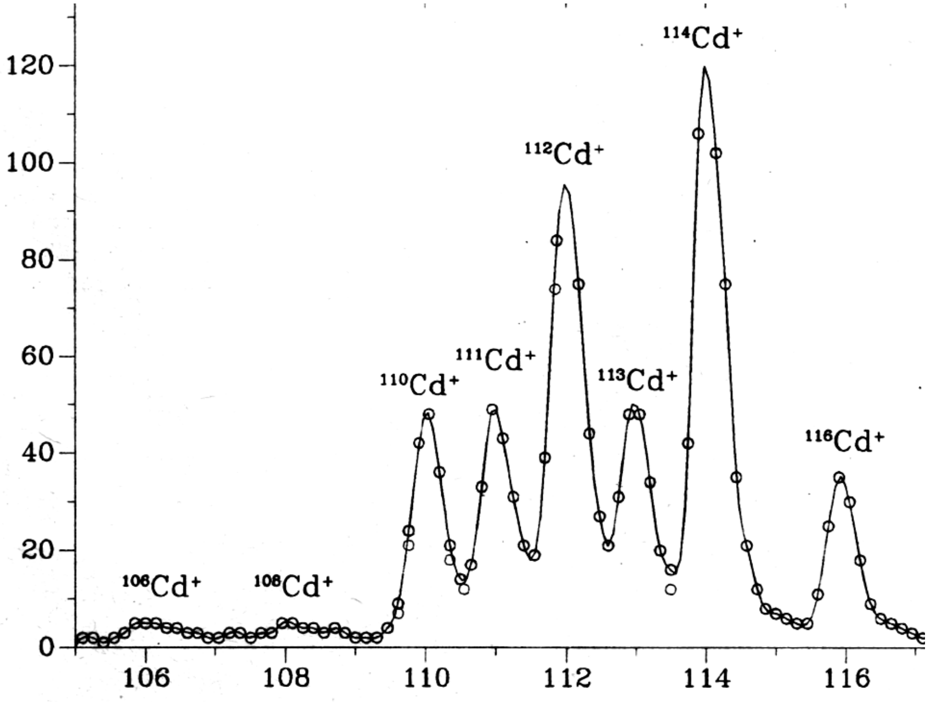

Figure 2. Cd mass spectum “fingerprint” after peak-fit identification (Simeonov et al., 1996).

In the present investigation some of the elements, with which the water solution is enriched, like Na, Mg and Fe, were excluded from consideration after a control analysis of drinking water samples. Likewise, the presence of Hg was observed very rarely, in more than 10% of all recorded spectra and only in the higher concentration solutions of the element of 1,000 and 500 ppb. The absence of Hg in spectra could be explained with its high volatility and escape from the water solutions in the evaporation process.

The last two elements from the enriched group, Ni and Cd, showed a constant presence in every laser shot and mass spectra and provided the necessary data for the purpose of the experiment, i.e. to prove the possibility to perform a quick semi-quantitative laser mass analysis of liquid samples at sub-ppm concentrations. As it was mentioned above, the solutions were prepared with concentrations of Cd from 1,000 to 62.5 ppb in a descending row of dilution with water, while the amount of Ni was kept constant at 2 ppm in every solution for the reason to provide a stable reference.

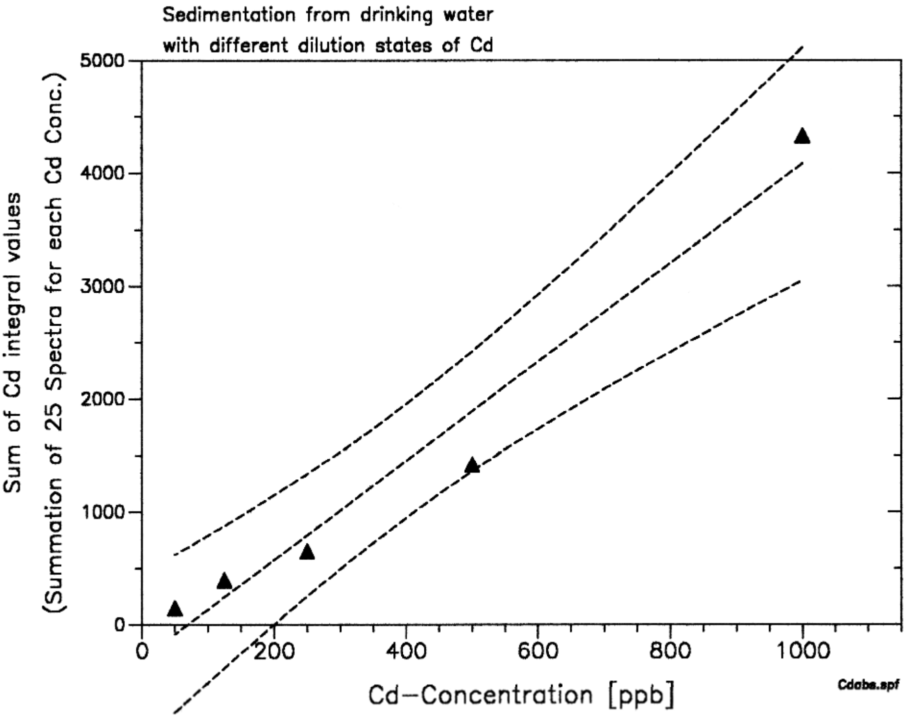

Figure 3. Laser mass analysis of targets obtained though evaporation and sedimentation from solutions of drinking water with different dilution states of Cd (Simeonov et al., 1996).

The semi-quantitative relations obtained are summarized in Figure 3. The abscissa gives the dilution states of Cd in ppb, while the ordinate gives the summarized values of Cd peak areas from 25 laser shot/mass spectra for each Cd concentration. As it is seen, the measured values follow the expected quantitative ratios in a rather satisfactory way as the observed relation is near to linear.

Another very important result of the experiments on preparation of solid state laser targets from water solutions concerns the quantitative dependence of the rates of sedimentation on the temperature of evaporation of the liquid constituent. On Figure 4 are presented the measured with the laser mass analyzer quantities from 25 laser shot/mass spectra on sedimentation targets of solutions with 1,000 ppb concentrations of Ni, Cd and Fe versus the temperature of evaporation.

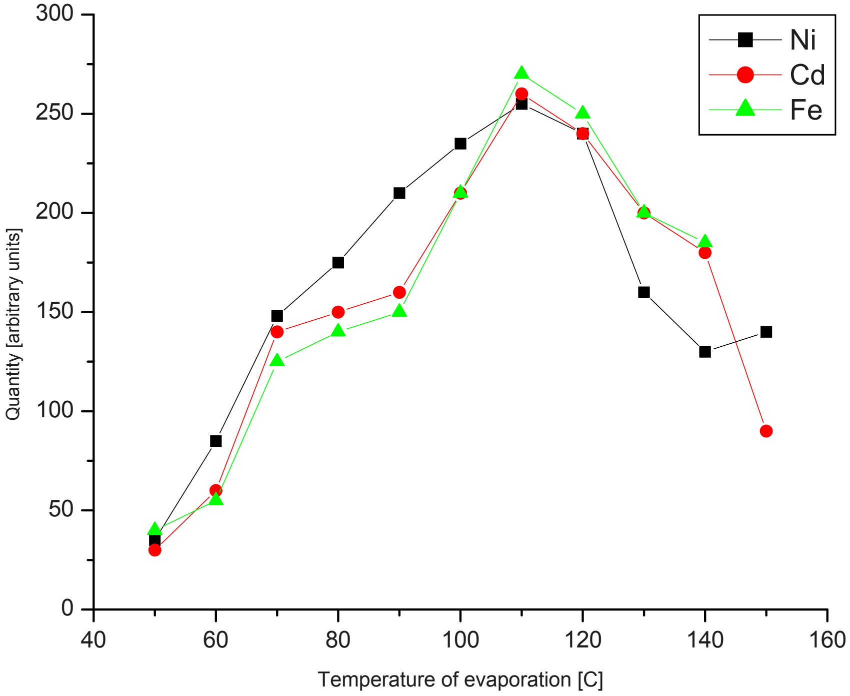

Figure 4. Dependence of the rates of sedimentation on the heating temperature for Ni, Cd and Fe.

The dependences of the rates of sedimentation on the temperature of evaporation, presented on Figure 4 are similar for the three elements and have two distinct regions. In the temperature region between 50 and 110°C the rate of sedimentation have an approximately linear character of increase because only the surface area of the liquid drop takes part in the evaporation process. The second region, which is above the boiling point, shows a clear descending trend as a proof of increase of the elements losses with heating temperature. In this case the process of evaporation of the liquid constituent is much faster and involves the whole volume of the drop as the elements are incorporated and leave the drop with the vapor bubbles.

5. Conclusions

The experiments carried out with a miniature laser mass spectrometer with targets, which are prepared by careful evaporation of water solutions, artificially enriched with dissolved metals, clearly proved the possibility to implement laser mass analysis in express in-field investigation of polluted aqueous environmental samples. The measured results are of semi-quantitative quality, which fully corresponds to the goals of *in-situ* measurements.

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A HEAVY METAL ENVIRONMENTAL THREAT RESULTING FROM COMBUSTION OF BIOFUELS OF PLANT ORIGIN

MARTA POGRZEBA[1]*, JACEK KRZYŻAK[1], ALEKSANDRA SAS-NOWOSIELSKA[1], WŁODZIMIERZ MAJTKOWSKI[2], EUGENIUSZ MAŁKOWSKI[3] AND ANDRZEJ KITA[4]
[1] *Department of Environmental Biotechnology, Phytoremediation Team, Institute for Ecology of Industrial Areas (IETU),*
6 Kossutha Street, 40-832 Katowice, Poland
[2] *Botanical Garden of National Center for Plant Genetic Resources, Plant Breeding and Acclimatization Institute - National Research Institute,*
5 Jeździecka Street, 85-687 Bydgoszcz, Poland
[3] *Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia,*
28 Jagiellonska Street, 40-032 Katowice, Poland
[4] *Department of Analytical Chemistry, University of Silesia,*
9 Szkolna Street, 40-006 Katowice, Poland

Abstract. Several assessments of the potential biomass supply in Europe show that the best means of biomass production are growing energy crops on agricultural land. Cultivation of energy crops on agricultural areas might lead to accumulation of heavy metals in plant tissues and reemission of contaminants into the atmosphere during combustion. The goal of the present study was to assess how soil contamination influences heavy metals accumulation in energy plant tissues. The current paper presents the results of screening of selected plants (*Spartina pectinata, Miscanthus sp., Helianthus tuberosus, Elymus elongatus*) conducted in uncontaminated region of Poland (North-Eastern part of the country) aimed at finding natural abilities to uptake small amounts of heavy metals and accumulate is in the plant tissue. Based on this screening, *Miscanthus* sp. was tested on heavy metal contaminated arable soil in Southern Poland. This species accumulates high amounts of metals what may cause high emission of contaminants during biomass combustion.

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* To whom correspondence should be addressed: mag@ietu.katowice.pl

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1. Introduction

Renewable energy sources (RES) are gaining an increasingly important role in the EU in reducing the emission of greenhouse gases. In Poland, which has been an EU member state since 2004, the policy on RES has for several years largely been guided by that on the EU level. Important landmarks concerning RES in Poland include the "Development Strategy for the Renewable Energy Sector". This document, accepted by the Polish Parliament in 2000, calls for a 7.5% contribution from RES in primary energy supply by 2010 and a 14% contribution by 2020 (Ministry of Environment, 2000; Ericsson, 2007). Biomass provides the largest reduction of carbon dioxide (CO_2) emission when it replaces hard coal and lignite, which are the most carbon-intensive fuels. In Poland, coal is the dominant fuel in heat and electricity production. Replacing coal by biomass usually also reduces the emission of sulphur dioxide (SO_2) (Ericsson, 2007).

Several assessments of the potential biomass supply in Europe show that the best means of biomass production are growing energy crops on agricultural land. This is particularly true for Poland, where a relatively large proportion (59%) of the total area is agricultural land, of which 76% is arable land (Ericsson et al., 2006). Regulations of Polish Ministry of Economy from 14 August 2008 appointed obligatory use of biomass of agricultural origin for co-combustion in power plants (Ministry of Economy, 2008). Till 2014, agriculture should reach 60% of total biomass used for heating and electricity. Within next years, agriculture has to combine production for food purposes on highest quality lands together with energy biomass production on lands useless for food and pasture purposes. On the basis of monitoring data and actual Polish law regulations (Ministry of Environment, 2002) more than 99% of total agricultural lands meets heavy metals content criteria.

In Silesian Voivodeship, the most industrialized Polish voievodeship, 5–10% of agricultural soils are contaminated by cadmium, lead and zinc. According to current law regulations more than 5% of agricultural soils contaminated by metals are located in southern part of Poland. It is connected with mining and smelting of Zn, Pb and Cd ores located in these regions (Siebielec et al., 2008). Cultivation of energy crops on agricultural areas might lead to accumulation of these metals in plant tissues and reemissions of contaminants into the atmosphere during combustion. Combustion of biomass introduces environmental risks due to content of heavy metals (especially Cd) in the combustion residues such as fly and bottom ashes (Dembiras, 2005). It is important to understand which ash fractions are formed and how will heavy metals behave during combustion process, because of its potential reuse as fertilizer or if contaminated, landfill in proper facilities (Vervaeke et al., 2006).

In order to cover the increasing demand for biomass, establishing of highly productive plantations of energy crops will be indispensable. Selection of the plants for energy crops should be done properly, taking into consideration specific soil-climatic conditions and technical capacity of the farmers.

Plant species cultivated for energy crops purposes should meet the following criteria:

- high biomass production,
- low costs of cultivation,
- easy harvesting technology,
- easy storage,
- good accommodation to local conditions (soil type, water, etc.),
- lack of contaminants (*e.g.* heavy metals).

The group of plants used as energy crops in Poland includes native and foreign species such as dendriform species of rapid rotation (willow, Salix sp.), perennial dicotyledonous plants (*e.g.* virginia fanpetals *Sida hermaphrodita,* cup plant *Silphium perfoliatum*) and perennial grass species: big blustem (*Andropogon gerardi),* miscanthus *(Miscanthus giganteus, M. sacchariflorus, M. sinensis),* switchgrass (*Panicum virgatum),* reed canarygrass *(Phalaris arundinacea),* eastern cordgrass (*Spartina pectinata*) (Majtkowski, 2007; Abassi and Abbassi, 2010). Different plant species produce different quantity of dry matter per unit of area of production, what determines the potential energy production capacity, or yield, of the available land area. Comparison between crop yield, caloric value (HHV) and energy yield of different plant species are presented in Table 1. *Miscanthus* sp. can give the highest crop yield, caloric value and energy yield per hectare compared to other energy plant species (Table 1).

TABLE 1. Crop yield, caloric value (HHV) and energy yield of different plant species (McKendry, 2002).

Biomass	Crop yield (t dm/ha)	HHV (MJ/kg,dm)	Energy yield (GJ/ha)
Wheat	7 grain + 7 straw (14 total)	12.3 (straw)	123
Poplar	10–15	17.3	173–259
Willow	10–15	18.7	187–280
Switchgrass	8	17.4	139
Miscanthus	12–30	18.5	222–555

The comparison between energy value of biomass from miscanthus, virginia fanpetals and willow in tones of coal, shows that miscanthus is the most promising species (Table 2).

TABLE 2. Crop yield caloric value and crop equivalent for selected plant species (McKendry, 2002).

Plant species	Yield (t dm/ha)	HHV (MJ/kg, dm)	Crop equivalent (in tones of coal)
Miscanthus	26	17	17.7
Virginia fanpetals	12	13	6.2
Willow	12	16.7	8

The goal of the present study was to assess how soil contamination influences on heavy metals accumulation in energy plant tissues. The current paper presents the results of screening of selected plants (*Spartina pectinata, Miscanthus* sp., *Helianthus tuberosus, Elymus elongatus*) conducted in uncontaminated region of Poland (North-Eastern part of the country) aimed at finding natural abilities to uptake small amounts of heavy metals and accumulate is in the plant tissue. Based on this screening, *Miscanthus* sp. were tested on arable soil contaminated in Southern Poland. This species accumulates high amounts of heavy metals what may cause high emission of contaminants during biomass combustion.

2. Materials and Methods

2.1. FIELD EXPERIMENTS

Experiments with energy plant species were performed on two sites. One of them, the uncontaminated site, is located in Białystok-Sowlany (North-Eastern part of Poland, Figure 1A). Selected species (*Miscanthus* sp., *Spartina pectinata* and *Elymus elongatus*) were assessed for content of heavy metals in plant tissues. Plants on Białystok-Sowlany site were sieved/seeded on 5 × 5 m plots. Grass seeds were sown in rows, with 25 cm interspaces, whereas seedlings of *Miscanthus* sp., *Helianthus tuberosus* and *Spartina pectinata* were planted with 0.5 m interspace. The experiment was performed during one growing season.

The second experimental site, contaminated arable land, is located in southern part of Poland in Silesian Voivodeship (‘Bytom site’, Figure 1B). It is in the outskirts of Bytom, industrial city about 15 km from Katowice in the proximity of a closed-down large lead/zinc/cadmium works consisting of the ore mining and smelting facilities. This metallurgical complex was in operation for more than 100 years and contributed significantly to pollution of local soils.

During the last 30 years the area was used for agricultural purposes. Recently the land has been used for grain crops farming, especially for wheat production.

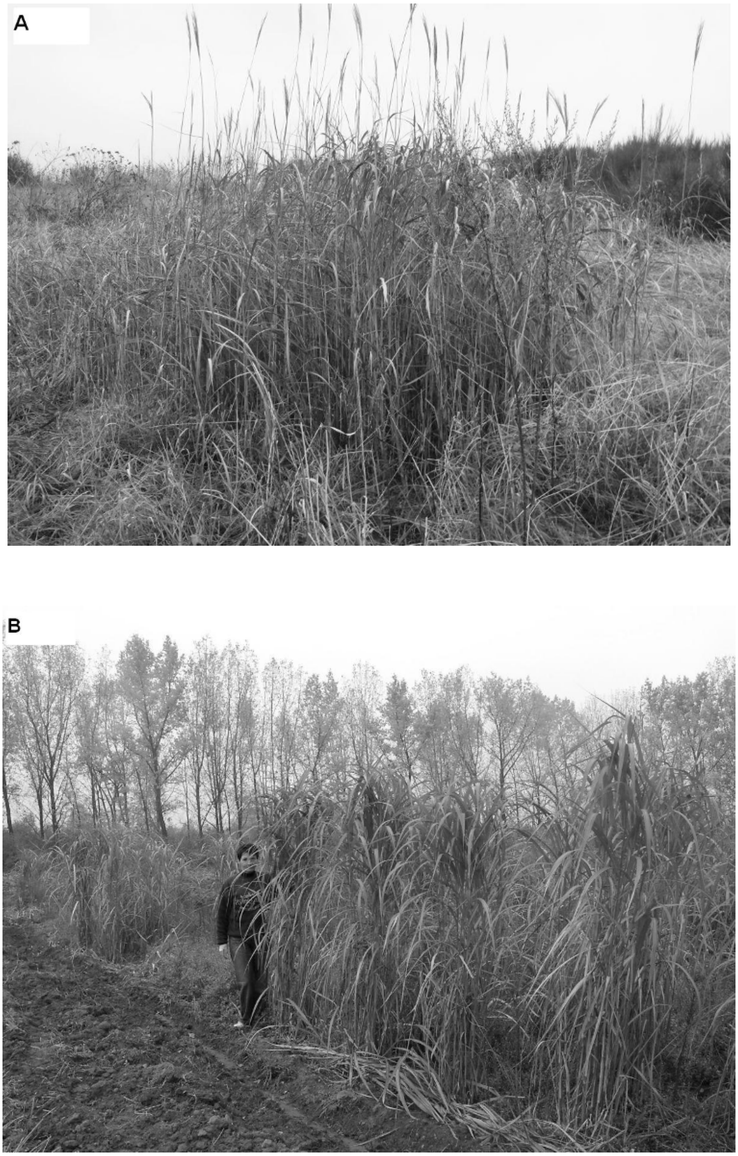


Figure 1. View of the experimental plots with *Miscanthus* sp. in ‘Białystok-Sowlany site’ (A) and in ‘Bytom site’ (B).

Crop yield achieved was rather influenced by fertilizing than environmental factors. The site is approximately 1 ha in size, but its flattest portion, where experimental plots were located is approximately 0.25 ha. Experimental field was divided into plots. The size of each subplot (4 × 4 m) with a buffer zone of 6 m separated the plots from each other. The soil was tilled to the depth of 20 cm and 49 seedlings of *Miscanthus* sp. per plot were planted. The experiment was performed during three growing seasons.

2.2. SAMPLING PROCEDURES

At the beginning of the field experiment, three composite soil samples per plot from the depth of 0–25 cm were taken and analyzed. Plant samples from ‘Białystok-Sowlany site’ were collected once at the end of growing season. During the 3 years field experiments at ‘Bytom site’, shoot samples of *Miscanthus* sp. were collected from each subplot at the end of each growing season.

2.3. ANALYSIS

Physico-chemical and chemical soil properties were analyzed using standard methods, as follows: pH (1:2.5 soil/KCl ratio) and total metal concentration (aqua regia extraction).

Plant samples were dried at 70°C for three days. Approximately 1 g of dried ground material was digested using concentrated nitric acid in a microwave system (MDS 2000, CEM, USA). Concentrations of metals were measured with flame atomic absorption spectrophotometry (Varian Spectra AA300) or by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Varian, USA). Data reported in this paper were processed using the computer software Microsoft Excel and Statistical for Windows (Statistica’99). A probability of 0.05 or less was considered to be statistically significant.

2.4. RESULTS AND DISCUSSION

2.4.1. *Soil properties*

Properties of soil from uncontaminated site in ‘Białystok-Sowlany site’ are presented in Table 3. In general, concentrations of Pb and Cd were typical for uncontaminated soils, while Zn exceeded Polish standards for arable soils (Ministry of Environment, 2002).

TABLE 3. Characteristics of soil used in field experiments located in uncontaminated site ('Białystok-Sowlany site').

Property	Value
pH (1:2.5 soil/KCl ratio)	6.47 ± 0.02
Total heavy metal concentration (extraction with aqua regia)	
Pb (mg kg^{-1})	8.73 ± 0.80
Cd (mg kg^{-1})	4.07 ± 0.44
Zn (mg kg^{-1})	385.5 ± 35.73

Values represent mean of three replicate samples ± SE.

Properties of the soil from contaminated site in Bytom (Upper Silesia Industrial Region) are presented in Table 4. The soil was classified as silty-clay loamy, polluted with Zn, Cd and Pb. Metals concentration exceed limits for arable soil (Ministry of Environment, 2002). The pH was almost neutral (Table 4).

TABLE 4. Characteristics of soil used in field experiments located in contaminated site (Bytom site).

Property	Value
pH (1:2.5 soil/KCl ratio)	6.79 ± 0.01
Total heavy metal concentration (extraction with aqua regia)	
Pb (mg kg^{-1})	547.0 ± 27.92
Cd (mg kg^{-1})	20.84 ± 1.17
Zn (mg kg^{-1})	2174.5 ± 103

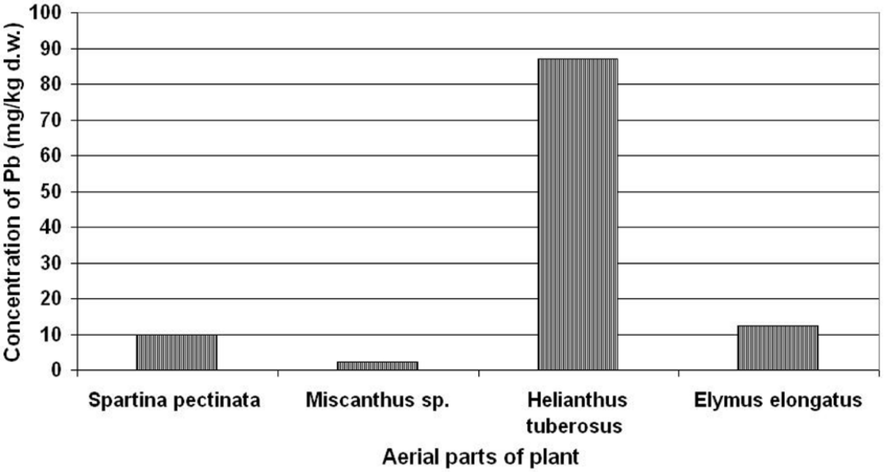
Values represent mean of three replicate samples ± SE.

2.4.2. *Concentration of metals in energy plant species*

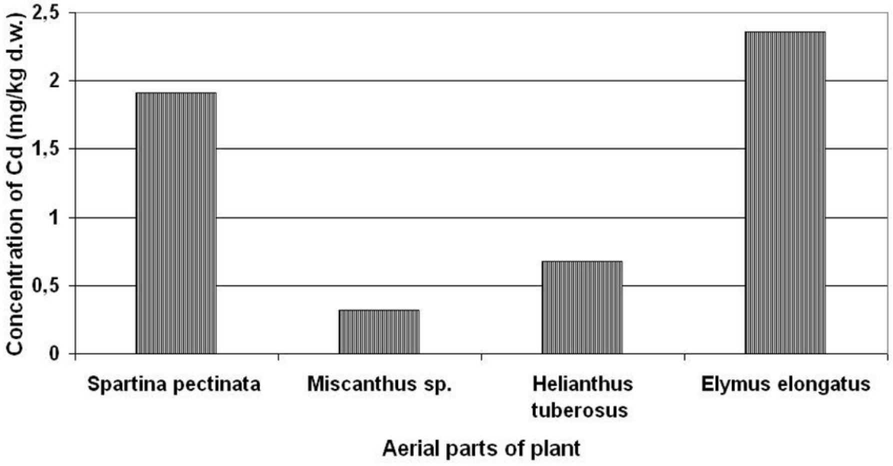
Experiments on selected energy plant species were conducted in North-East part of Poland, region which is not under industrial pressure. Concentration of metals in tissues of energy plants (mg/kg d.w.) grown in uncontaminated 'Białystok-Sowlany site' are shown in Figure 2. Lowest metal accumulation was found in *Miscanthus* sp. shoots, in comparison with other species. Lead accumulation in *Spartina pectinata* and *Elymus elongatus* were at the same level (Figure 2a). In case of *Helianthus tuberosus*, Pb accumulation was 8–40 times higher than in shoots of other species. Low concentrations of Cd were found for *Miscanthus* sp. and *Heliantus tuberosus*, while in shoots of *Spartina pectinata* and *Elymus elongatus* concentrations of these metals were 2–4 times higher compared to other species (Figure 2b, c). In case of Zn, lowest concentrations were found in *Miscanthus* sp. and *Helianthus tuberosus* shoots. In other plant species (*Spartina pectinata* and *Elymus elongatus*), accumulation of Zn was 3–13 times higher, in comparison with the lowest accumulating species.

On the basis of obtained results it was found, that *Helianthus tuberosus* has natural abilities for Pb uptake from the soil, while *Spartina pectinata* and *Elymus elongatus* are accumulating high amounts of Zn and Cd. High amounts of toxic elements uptake by plants may cause energy crop contamination, but on the other hand it can be used for phytoremediation purposes. The lowest concentration of all metals was found in *Miscanthus* sp. shoots.

a)



b)



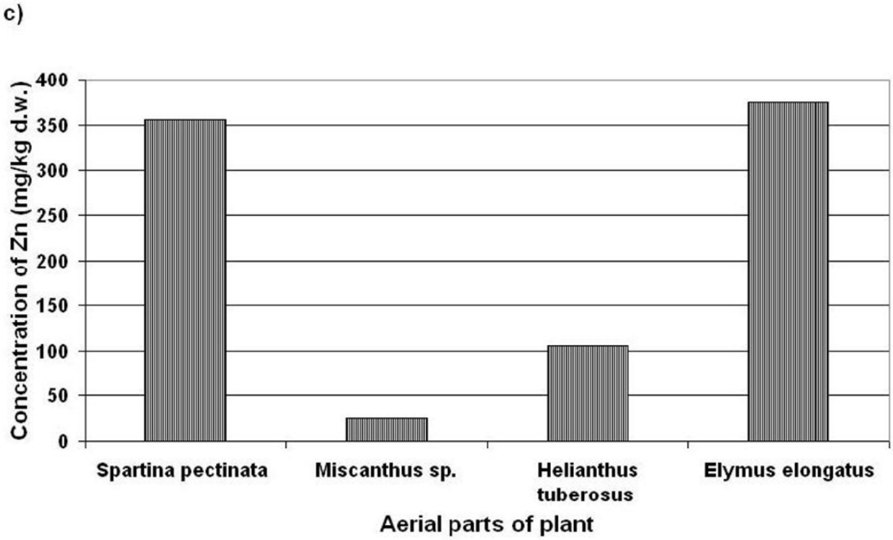
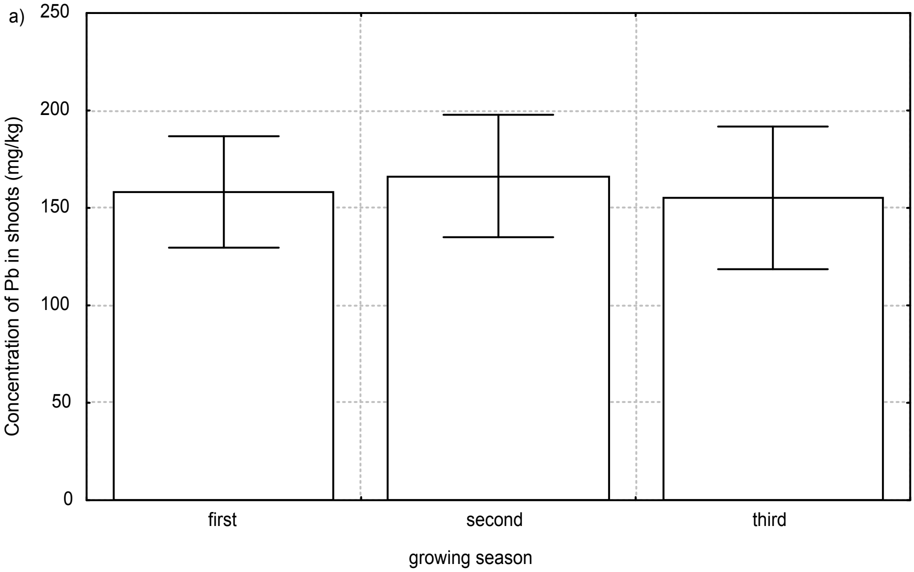
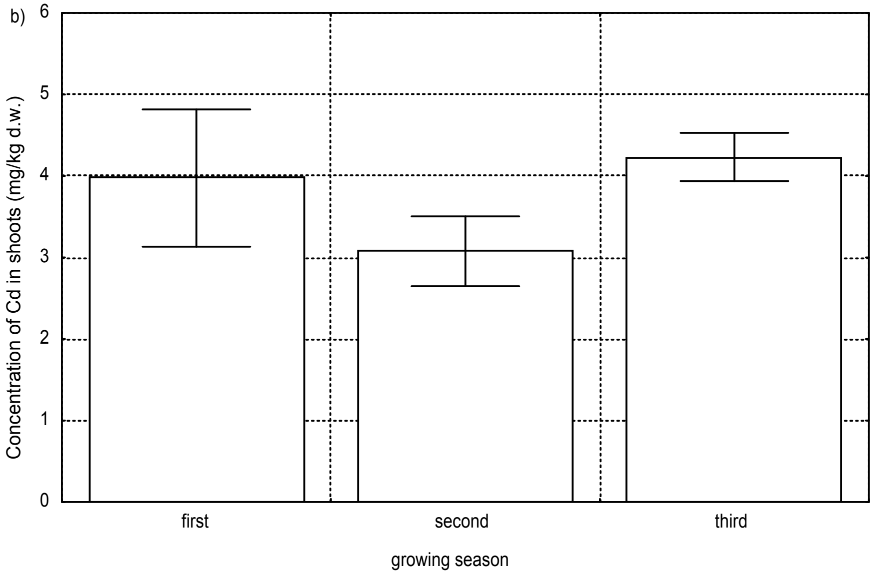


Figure 2. Concentration of metals in tissues of energy plants (mg/kg d.w.) grown in 'Białystok-Sowlany site': a) Pb; b) Cd; c) Zn.

Experiments were also conducted on the soil contaminated with heavy metals ('Bytom site'). One of the goals of these experiments was the assessment of crop contamination. Based on results of plant screening the best plant (*Miscanthus* sp.) was chosen for this experiment. Accumulation of Pb, C Cd and Zn in shoots of *Miscanthus* sp. is presented in Figure 3.





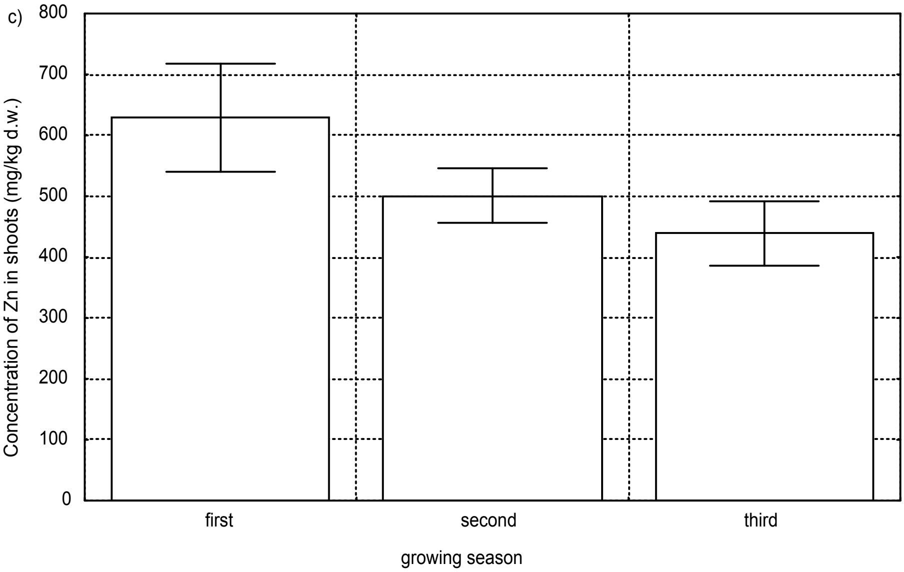


Figure 3. Concentration of metals in shoots of *Miscanthus* sp. grown on ‘Bytom site’: Pb (mg/kg d.w.); b) Cd (mg/kg d.w.); c)Zn (mg/kg d.w.).

During three growing seasons the concentration of Pb in shoots was on the same level (Figure 3a). High level of Pb in biomass was related to soil contamination by this metal. Cadmium, concentration in *Miscanthus* sp. tissues in the end of first and third growing season was at the same level, whereas in second growing season concentration of the metal was lower. Cd concentration in biomass was in the range of 3.08–3.97 mg/kg d.w. (Figure 3b). During 3 years of experiment, concentration of Zn in grass biomass was diminishing, resulting in lowest Zn concentration in third year of the experiment. It was found that, high concentration of Zn in *Miscanthus* sp. shoots was related to high soil contamination with this metal (Figure 3c). Experiments, conducted by

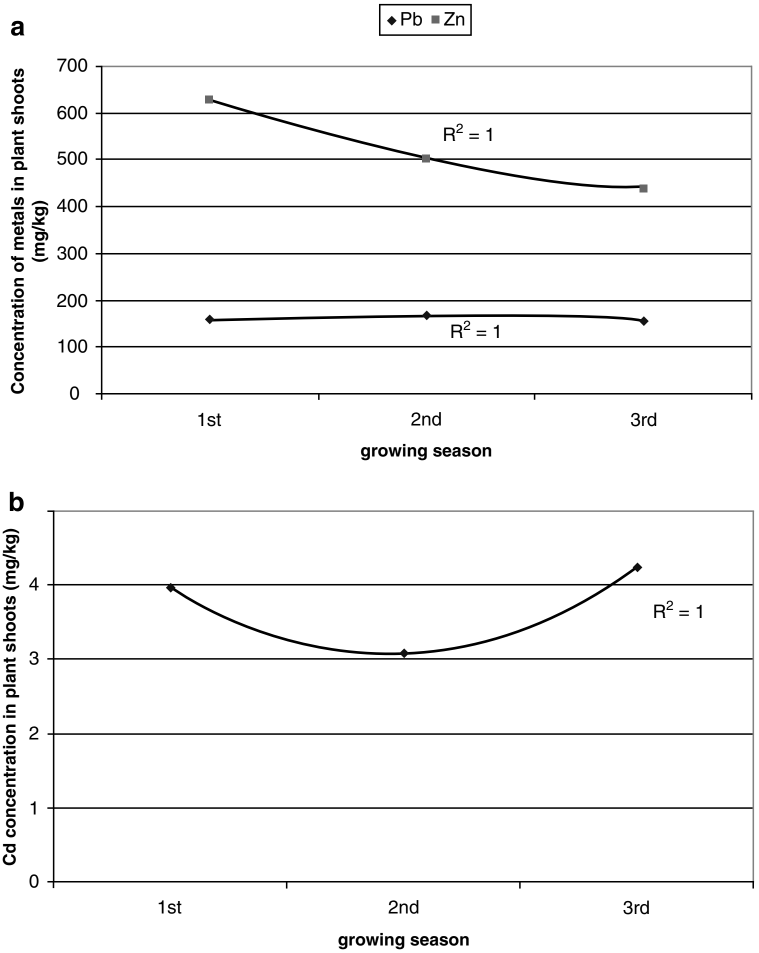


Figure 4. Trends in metals concentrations in *Miscanthus* sp. shoots: a. Concentration of metals; b. Cd concentration.

Meers et al. (2010) with monocotylenoides plant (*Zea mays*) as energy crop on agricultural contaminated area, showed 4–10 times lower metal concentration in plant tissues.

Although during experiments with *Miscanthus* on uncontaminated soil lower metal accumulation from tested plant species was obtained, the same species cultivated on the soil contaminated with Pb, Zn and Cd accumulated substantial amount of these elements. The same relation was observed by Arduini et al. (2006) in case of *Miscanthus* sp. grown with increasing Cd concentration in hydroponics experiment.

Positive trend in diminishing of zinc concentrations in *Miscanthus* sp. tissues was found (Figure 4a). Diminishing of zinc concentration was observed in regular crops. Lead content was on the same level in three crops (Figure 4a), whereas cadmium, after small decrease of it's concentration in the second crop, exceed the value of 4 mg/kg in the third crop (Figure 4b). However trend lines should not be the final arbiter, but by using them closer attention may be put on data variability.

3. Conclusions

Between plant species grown on uncontaminated soil the least Pb, Cd and Zn accumulation were found for *Miscanthus* sp. However in case of contaminated soil, this species accumulates high amounts of metals and it may cause high emission of contaminants during biomass combustion. In this case the determination of the safe levels of metals in plant tissue and in soils for *Miscanthus* sp. and other energy crop plantations should be assessed, as well as identification of the safe ways for crop management. Setting-up a clear labeling system for the crop and soil, aimed at production of an environmentally sound material should be arranged. To diminish metal elevated levels in *Miscanthus* sp. tissue using soil remediation towards decreasing of bioavailable amounts of metals should be done. Identification of the safe levels of heavy metal content in combusted biomass should be the main issue of scientific work nowadays.

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HEAVY METALS AS PERSISTENT PROBLEM FOR BALKAN COUNTRIES

MIHAIL KOCHUBOVSKI*
Institute of Public Health of the FYRepublic of Macedonia
50 Divizija No. 6, 1000 Skopje, FYRepublic of Macedonia

Abstract. The objective of the study was meta-analysis of the soil pollution with heavy metals and related adverse health effects on exposed population in the Balkan's region. Heavy metals are naturally occurring elements, and are present in varying concentrations in all ecosystems. There are a huge number of heavy metals. They are found in elemental form and in a variety of other chemical compounds The main anthropogenic sources of heavy metals are various industrial processes, mining, foundries, smelters, combustion of fossil fuel and gasoline, and waste incinerators. The major heavy metals of concern to WHO and EMEP are Hg, Cd and Pb, because they are the most toxic and have known serious effects on e.g. human health. Soil contamination is a problem discussed as a degraded process which importance exceeds agricultural area. In 2007 CERCLA Priority List of Hazardous Substances-ATSDR has been ranked several heavy metals by their toxicity. In the paper were presented some results from Macedonia, Albania, Bulgaria, Serbia and Turkey.

Keywords: Heavy metals, soil pollution, environmental-health, population

1. Introduction

Heavy metals are naturally occurring elements, and are present in varying concentrations in all ecosystems. There are a huge number of heavy metals. They are found in elemental form and in a variety of other chemical compounds. Those that are volatile and those that become attached to fine particles can be widely transported on very large scales. Each form or compound has different properties which also affect what happens to it in food, water and soil, and how toxic it is. Human activities have drastically changed the biochemical cycles and balance of some heavy metals. The main anthropogenic sources of heavy

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* To whom correspondence should be addressed: kocubov58@yahoo.com

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metals are various industrial processes, mining, foundries, smelters, combustion of fossil fuel and gasoline, and waste incinerators. The major heavy metals of concern to EMEP are Hg, Cd and Pb, because they are the most toxic and have known serious effects on e.g. human health. Environmental exposure to high concentrations of heavy metals has been linked with e.g. various cancers and kidney damage. There are considerably more measurements data on Hg, Cd and Pb in Europe than for other metals (Nriagu and Pacyna, 1988).

Soil contamination is a problem discussed as a degraded process which importance exceeds agricultural area. It is a part of the global disturbance in the ecosystem in transport of energy and substances, and is a main effect of the soil degradation. Heavy metal contamination of soils (and especially soils with low pH) leads up to serious problems in the trophyc food chain. It is well known that the availability of a number of trace elements becomes greatest at low pH and the reasonable result is that plants absorb more quantities of toxic elements (Benkova, 2005).

Absorption and accumulation of metals in plants are complicated processes, defined by the behaviour of the element, depending on soil characteristics and the properties of the biological reagents. That is why the regular investigations of these processes have to be a part of the environmental monitoring (Japenga et al., 2005).

Heavy metals deposited on ground can contaminate crops and through the food chain (irrigation, ingestion) as well inhalation of dust and dermal absorption from soil could enter human body and impact its health. Bioaccumulation of heavy metals by fish from water and sediment and through food chain can lead to adverse health effects to humans.

According to the CERCLA Priority List of Hazardous Substances by ATSDR (Agency for Toxic Substances & Disease Registry) (ATSDR, 2007) several heavy metals are **prioritized based on a combination of their frequency, toxicity, and potential for human exposure** and possible adverse impact to human health:

▪	Arsenic	1st rank/N
▪	Lead	2nd rank
▪	Mercury	3rd rank/I
▪	Cadmium	7th rank/I
▪	Cobalt	49th rank
▪	Nickel	53rd rank
▪	Zinc	74th rank
▪	Chromium	77th rank/N
▪	Manganese	117th rank/N
▪	Copper	128th rank

N = substances that occur in nature

I = substances from industrial or domestic sources

According to the above mentioned list it is obvious that arsenic, lead mercury and cadmium are most toxic and all of them are present in soil from Balkan's area.

2. Material and Methods

In the frame of the study has been performed meta-analysis of published papers in Peer Reviewed Journals regarding the soil pollution with heavy metals and related adverse health effects on exposed population in the Balkan's region.

3. Aim

The objective of the study was meta-analysis of the soil pollution with heavy metals and related potential adverse health effects on exposed population in the Balkan's region. In the paper are presented some results from Macedonia, Albania, Bulgaria, Serbia and Turkey.

4. Results and Discussion

In the Study on capacity development for soil contamination management related to mining in the Republic of Macedonia - Master Plan of capacity development for soil contamination management has been made a List of Hot Spots of soil contamination related to mining in Macedonia (Mitsubishi, 2007). Republic of Macedonia is very well known about his mining activities and on Table 1 is presented Mine/Companies, their location, heavy metals and status of operation.

Methodology of the investigation in the study was done by taking soil samples according to standard procedure and analysis of the following heavy metals with ISO methods:

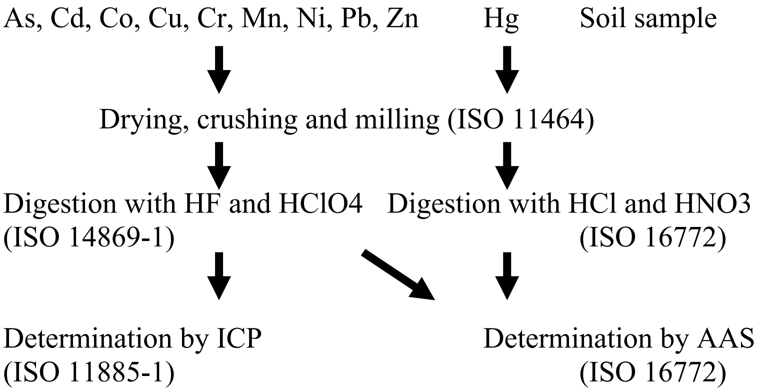

TABLE 1. List of Hot Spots of Soil Contamination Related to Mining in Macedonia.

Level of environmental risk	Mine/company name and location	Heavy metals operating/closed
High	Zletovo Mine, Probistip	Pb, Zn, operating
	Sasa Mine, Macedonian Kamenica	Pb, Zn, operating
	MHK Zletovo, Smelter, Veles	Pb, Zn, sleeping
	Bucim Mine, Radovis	Cu, open pit, operating
Medium	Lojane Mine, Kumanovo	Cr, As, Sb, closed
	Silmak Ferro-silicon plant, Jegunovce	Ferro-silicon, operating
	Toranica Mine, Kriva Palanka	Pb, Zn, operating
	Makstil iron and steel plant, Skopje	Fe, operating
	Krstov Dol Mine, Kriva Palanka	Sb, closed
Low	REK Suvodol Mine, Bitola	Lignite, operating
	REK Oslomej – ESM, Kicevo	Lignite, operating
	Feni Industry, Ferro-nickel Smelter, Kavadarci	Ferro-Ni, operating
	Rzhanovo Mine, Kavadarci	Ni, operating

Characterization of the risk by heavy metals in the study was done by taking into account the following variables:

- Pathway of soil - human health (agricultural products);
- Time of exposure (365 days/8 h-agricultural area, 12 h/day-tailing dams or 24 h/day-residential area);
- Year of inhabitation;
- Body weight;
- Intake of soil; intake of Groundwater;
- Breathing volume;
- Soil mechanical condition.

Stafilov et al., 2010a in their study have collected samples of natural surface soils in the town of Kavadarci and the surrounding region according to the European guidelines for soil pollution studies (Theocharopoulos et al., 2001; Darnley et al., 1995; Reimann et al., 2008), and also according to (Šajn, 2003, 2005, 2006). The complete investigated region (360 km^2) was covered by a sampling grid 2 x 2 km^2, but in the urban zone of Kavadarci and around the ferronickel smelter plant (117 km^2), the sampling grid was denser, 1 x 1 km^2. Altogether 344 soil samples were collected from 172 locations. At each sampling point, soil samples were collected at two depths, topsoil (0–5 cm) and subsoil

(20–30 cm). The possible organic horizon was excluded. The mass of such a composite sample was about 1 kg.

The soil samples were air-dried indoors at room temperature for about 2 weeks. Then they were gently crushed, cleaned from extraneous material and sifted through a plastic sieve with a 2 mm mesh (Miesch, 1976; Salminen et al., 2005). The shifted mass was quartered and milled in an agate mill to an analytical grain size below 0.125 mm.

Mass spectrometry with inductively coupled plasma determination of 36 elements was performed in the laboratory of ACME Ltd. in Vancouver, Canada, after aqua regia digestion (mixture of HCl, HNO3 and water at 950°C—1DX method). The following 31 elements were analyzed: Al, As, Au, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, La, Mn, Na, Mg, Mo, Ni, P, Pb, Sb, Sc, Sr, Th, Tl, Ti, U, V, W and Zn.

Critically high concentrations (The new Dutchlist (http://426 www.contaminatedland.co.uk/std-guid/dutch-l.htm) are related primarily to high concentrations of Ni and Cr that are found both in topsoil, subsoil and in the sampling points from the SW, W and NW part of the investigated region. The highest concentrations of Cr and Ni are found in sampling points near the village Debriste, SE from the village Vozarci and W from the hydropower plant Tikves. The three sampling points are located on the outcropping Paleozoic serpentinites (inner parts of the Vardar zone). Taking into account the fact that the concentrations of these elements are higher in subsoil than in topsoil, it can be concluded that the occurrence is natural. High, sometimes critical content of Cr and Ni in the zone of Eocene flysch is already proven in numerous researches from Macedonia (Sayegh-Petkovsek et al., 2007) and other Balkan countries (Šajn, 2003, 2005; Alijagic, 2008; Šajn et al., 2006).

It was found that the critically high concentrations are related primarily to high concentrations of Ni and Cr in the sampling points from the western part of the investigated region (Table 2). The concentrations of these elements are higher in subsoil than in topsoil, it can be concluded that the occurrence is natural. The high content of Cr and Ni is found in the zone of Eocene flysch.

This fact indicates that the ferronickel smelter plant situated in this region, in spite of the obvious environmental pollution by ore dust, has not contributed significantly to the measured content of these elements, due to the their high content in the background.

Pančevski et al. (2006) in their Study have investigated copper content in the soil of Veles with historical pollution from lead Smelter Plant. Samples of natural surface soil in the town of Veles and surrounding region were collected according to the European guidelines for soil pollution studies (Theocharopoulos et al., 2001).

TABLE 2. Heavy metals in soil in Kavadarci area (mg/kg).

Heavy metal	Minimum	Maximum	Mean EU level*
As	5.1	13	70
Pb	14	33	23
Cd	0.2	0.6	0.15
Ni	42	150	18
Cr	31	110	60
Mn	520	1,100	500
Zn	40	78	52
Cu	18	52	13

* Mean (EU) European topsoil average (Salminen et al., 2005. Geochemical Atlas of Europe. Part 1. Background information, methodology and maps. Geological Survey of Finland, Espoo.

The surroundings were sampled on a 1 × 1 km grid; the town on a 500 × 500 m; and in the polluted areas on a 250 × 250 m grid. A total of 201 samples were collected over the area of 33 km^2; about 20 samples of this number were collected in the direct vicinity of the plant. Soil was sampled from the topsoils (0–5 cm). The possible organic horizon was excluded. A sample represents the composite of material collected in the sample point and in four points on 10 m around it towards N, E, S, and W.

A systematic study of copper distribution in surface soil over of the Veles region, known for its lead and zinc industrial activity, was undertaken. A total of 201 soil samples were collected according to a dense net (0.5 km) in urban and less dense net (1 km) in rural areas.

Copper was determined by flame atomic absorption spectrometry (FAAS) using microwave digestion technique with two different types of solvents: aqua regia (HCl and HNO_3) and the mixture of strong acids (HNO_3, HCl, and HF). So far the same soil samples were subjected to reactor non-destructive multi-element instrumental neutron activation analysis (INAA), it served as a reference analytical technique for bulk copper determination. The results obtained by two methods of FAAS and INAA are discussed. GIS technology was applied to reveal the areas most affected by copper contamination. It was found that the content of copper in soil samples around the lead and zinc smelter plant is the highest and reaches 1,800 mg/kg. Copper content in surface soil all around the town of Veles exceeds maximum permissible level for urban surface soil.

Elevated copper content in some rural areas of the Veles region most likely could be explained through using copper containing fungicides for agricultural needs.

Heavy metals contamination around the Lead Smelter Plant in Veles area has been investigated in study of Stafilov et al., (2010b). Copper average content in soil for the whole investigated region of Veles is 49 mg kg^{-1}, while the average content for the surroundings is 33 mg kg^{-1}.

Range of copper concentrations is from 10 to 1800 mg/kg. For Veles town the median is 49 mg kg^{-1}, showing that soil in the surroundings of the town of Veles also characterized by relatively high copper content. This is especially important for the soils around the lead and zinc smelter plant were the content of copper in more than 20 soil samples is higher than 100 mg kg^{-1} (in some cases more than 500 mg kg^{-1}) with maximal copper content of 1800 mg/kg in sampling point.

Comparing these results with the maximum permissible levels for soil in some European countries (for example, in the Netherlands were the limits is 36 mg kg^{-1}, or Germany with the limit of 50 mg kg^{-1} (Kabata-Pendias and Pendias, 2001) the soils of the large part of Veles region are contaminated with copper.

The obtained results of the study of spatial distribution of different chemical elements in the surface soil over of the Veles region in the Republic of Macedonia show that the content of elements such as As, Au, Cd, Cu, Hg, In, Pb, Sb, Se, Zn in the soil samples around the lead and zinc smelter and in the adjacent part of the town of Veles is much higher than in those collected in the surrounding areas due to the pollution from the plant (Table 3).

TABLE 3. Heavy metals in Veles area (mg/kg).

Heavy metal	Average	Cultivated soil	Uncultivated soil	Urban soil	Polluted soil
As	9.8	7.8	11	13	15
Au	11	10	10	12	14
Cd	7.7	6.1	4.8	12	32
Cu	44	38	41	52	69
Hg	0.25	0.20	0.18	0.36	0.50
In	0.25	0.22	0.22	0.31	0.65
Pb	220	170	140	340	800
Sb	2.3	1.6	1.7	4.5	6.7
Se	0.25	0.19	0.19	0.42	1.3
Zn	280	210	180	460	1,100

In prospective-ecological study from January 2001 till January 2002 it has been assessed the relationships between the level of lead in the soil around the Smelter Plant for lead and zinc in Veles and lead blood levels of children living nearby (Kochubovski and Gjorgjev, 2003).

Environmental monitoring of lead and lead blood levels in children at the age of 10 years, who live in Veles with a stationary source of lead, have been compared with those living in place without a stationary source of lead in village Ivankovci.

Lead blood levels in children living in environmentally clean area in v.Ivankovci with lead levels into the uncultivated soil of 36.32 mg/kg (36% from MAC-maximum admissible concentration) have been registered in a range ($x = 18.2$ μg/dl ± 17.935).

The levels of lead from the surface of uncultivated soil near to the stationary source of lead have been increased compared to the control area (191 mg/kg almost 2 fold higher than the MAC), as well the average concentration of lead in blood ($x = 37.27$ μg/dl, ± 11.56).

With Student t-test for big independent samples, it has been registered significant difference ($t = 5.74$; $p < 0.05$) related to blood lead levels among the sample of children from Veles ($n = 203$) and v.Ivankovci ($n = 31$). It can be concluded that there is a possible relation between the level of lead into the soil and blood lead levels from the exposed children.

A new study (Directive 2000/60/EC) has indicated that metallic pollutants in river basins have more sources than other dangerous substances. Sources include storm water, industrial effluents, treated effluents, agricultural drainage, sediments, mining drainage and landfills.

The EU Water Framework Directive requires Member States to take actions to achieve good chemical and ecological status of surface and ground water by 2015. It has identified 33 priority substances plus 8 other pollutants that are harmful to ecosystems and aquatic systems and include cadmium, mercury, lead and nickel. As a starting point, Member States are required to identify all sources of emissions affecting water quality in river basins.

The study identified and assessed the main human sources of metallic substances in receiving waters and summarised research findings in this area.

The effects of storm water on metal levels in water have increased with urbanisation. Levels of cadmium, lead, copper and zinc in storm water can be as high as those found in raw sewage and wastewater treatment plant (WWTP) effluent. The atmosphere, traffic and building materials are the most significant sources of metals in storm water. Traffic wastes from sources, such as brake linings and road dust, generate substantial metal levels in water run-off from roads, particularly zinc but also lead and cadmium. Building materials used in roof coverings affect concentrations of mainly copper, lead and zinc in roof run-off.

The influence of metals due to effluents from industry will vary with country because each Member State implements its own regulations on treatment before discharge into receiving waters.

Effluents from WWTPs contribute significantly to a wide range of metal concentrations. The source of these can be household products, commercial effluents (especially car washing) and drinking water. Although WWTPs reduce total levels of metals, a proportion of dissolved metals remains untreated, particularly nickel. Metal levels in drinking water are influenced by geological conditions, plumbing systems and purification processes. The sewage system itself gives rise to metal inputs due to copper sewage pipes and, if left for a long time, the sewage sediments can cause metals to remix in the water.

Agriculture is another source of metals. Phosphate fertilisers release both cadmium and zinc, whilst sewage sludge used as a fertiliser may be a source of nickel, cadmium and zinc.

The research listed several additional factors that can increase metal concentrations. Sediments are an integral part of water systems but also a potential source of metal pollutants. The contribution of sediments to metal concentrations in waters has been estimated to be 20% for cadmium, 30% for copper and 10% for zinc. The mining industry can also emit metals into water from mine waste, such as tailings and mine water. Landfills contribute to metal concentrations due to rainwater flowing through waste that contains metals. Lastly, sporting activities such as boating, fishing and shooting, also contribute to metal concentrations.

The study has outlined several important sources of metallic pollutants. Further research is needed to evaluate the relative contributions of each source on a case-by-case basis and to investigate the influence of societal and environmental conditions, for example, by collecting data on household practices and rainfall specific to catchment areas.

Sundseth et al., 2010 have assessed the annual damage costs due to IQ loss from ingesting methyl mercury. It was estimated to be US$2.9 billion (€2.1 billion) caused by non-intentional emissions of mercury (e.g. as a by-product from power plants, smelters and kilns) and US$0.8 billion (€0.6 billion) from emissions due to its intentional use in products.

The corresponding damages from inhalation of mercury were estimated to be US$2.9 million (€2.1 million). This is a small fraction of the costs associated with eating food contaminated with mercury. However, the researchers point out some population groups, such as small-scale gold miners, would be particularly affected and could suffer more serious health problems with correspondingly much higher damage costs.

By 2020, the study suggests the introduction of emission reduction measures could reduce the damage costs to society under the EXEC scenario compared with the status quo by more than half and produce societal benefits of around US$1.8 billion (€1.3 billion) annually. Additional benefits of US$0.4 billion (€0.3 billion) could be achieved under the MFTR scenario.

Adverse health effects from the exposure to mercury was assessed in the study of Sezgin et al., 2010. This article presents a 36-year-old case admitted to emergency department (ED) due to exposure to metallic mercury. She had a three-day history of abdominal pain, diarrhoea and fever. One week ago her daughter had brought mercury in the liquid form from the school. She had put it on the heating stove. One day later. Her 14-month old sister got fever and died before admission to the hospital. Her blood pressure was 134/87 mmHg; temperature 40.2°C; heart rate 105 bpm and regular; respiration 18 bpm; O2 saturation 96%. Nothing was remarkable on examination and routine tests. As serine or urinary mercury levels could not be tested in the city, symptomatic chelation treatment with N-acetyl cysteine (NAC) was instituted with regard to presumptive diagnosis and history. At the 7th day of admission she was discharged without any sequelae or complaint. At the discharge day blood was drown and sent for mercury levels which turned out to be 30 μg/dl (normal range: 0–10 μg/dl).

WHO in Health and Environment in Europe: Progress Assessment in 2010 has revealed main sources, potential health problems from exposure and provisional tolerable weekly intake for arsenic, lead, methyl mercury and cadmium (Table 4).

TABLE 4. Main sources, potential health problems from exposure and provisional tolerable

Heavy metal	Main sources	Primary health concerns	PTWI (mg/kg body weight)
Arsenic group 1/IARC	Contaminated food/water, some paints	Neurological and developmental disorders	0.015
Lead group 2B/IARC	Air, water, fuels, marine life	Neurological and neurodevelopmental disorders; organ damage	0.025
Methyl mercury	Fish/seafood	Neurological and developmental disorders	0.0016
Cadmium group 1/IARC	Contaminated food	Kidney damage, low birth weight, spontaneous abortion	0.007

It has been studied the contamination of Cu, Zn, Pb, and Cd in soils under oak ecosystems of urban, suburban, and rural regions in Sofia, Bulgaria (Doichinova et al., 2006). The urban sampling sites are located in the large forest parks of Sofia under oak ecosystems, while the others are located between the centre of the town and the surrounding mountains and are also from oak ecosystems. The concentrations of Cu, Zn, Pb, and Cd in soils were measured for identifying the sources and degree of contamination, and calculating the concentration coefficients (Hc) and enrichment factors (EF). The result of applying principal component analysis (PCA), showed that Cd and Pb accounted for the anthropogenic pollution and could be inferred as its tracers, whereas Cu is located mainly in the parent material. The Zn concentration levels were controlled both by its original content in the parent material as well as by anthropogenic pollution. The results obtained for the city forest parks allow for their successful use for recreation purposes.

The investigation was related to application and adopting of monitoring set in areas with expressed local contamination and high impact of diffuse contamination (one of so called "hot spots" in Bulgaria). The area is located around the actively functioned gold extracting factory "Chelopech Mining" EAD. The results showed that the content of heavy metals exceeded the accepted maximal permissible concentrations in respect to As (31.7 mg/l), Cd (0.6 mg/l), Cu (118.5 mg/l), Zn (98.1 mg/l), and Pb (39.5 mg/l) in randomly spread samples. The soil acidification process was established (Dinev et al., 2008; Novosielska and Dinev, 2005).

The metallurgical complex of Elbasan is the largest and most important one in the country but at the same time represents a source of significant heavy metal contamination in the wider watershed Shkumbini River (Sallaku et al., 2009). This study aimed to determine the degree of soil contamination by Cu, Zn and Cd, with respect to distance from the metallurgical complex and determine various metal fractions for selected soil samples. Samples (80) from surface soil (0–20 cm) were collected at 1, 2, 3, 4, 5, 6 and 7 km distance from the metallurgical plant. The data indicated that the soils around the plant are highly polluted up to 15 km away from the plant with Cd, Zn and Cu (50–159; 86–147; 0.76–2.25 mg/kg soil, respectively). A sequential extraction technique used to characterize bonding of metals to the soils showed that organic matter, carbonate and poorly crystalline Fe oxides and tightly bound residual fractions contained >60% of the total Cd, Zn and Cu. Exchangeable Cd was a significant fraction, averaging from 30 to 40% of the total present. Although amounts of organic matter and Fe oxides were of obvious importance in influencing this distribution, there was little variation in different fractions found in this study. Results from this study would help to implement appropriate soil-management techniques to limit mobility and plant availability of heavy metals minimizing their transfer into the food chain.

Thirty-eight soil samples from six soil sites representative of different parent materials of Tirana region were sampled and analysed for basic characteristics and also for the total form of heavy metals after extraction with aqua-regia (Gjoka et al., 2008).

The aim of this research was to establish the background and reference values of heavy metals for agricultural soils of this region. Results showed that the total Cd, Cr, Ni, Pb, Zn and Cu contents of the study soils varied widely with respective mean values of 0.31, 168.6, 291.89, 17.6, 93.5, 42.3 mg kg^{-1}. The background values of total metal contents based on the 90th percentile criterion were (mg kg^{-1}): Cd (0.8); Cr (227.6); Ni (455.6); Pb (19.9); Zn (113.3) and Cu (51.9). While the reference values were (mg kg^{-1}): Cd (0.7); Cr (113.7); Ni (41.9); Pb (85.5); Zn (151) and Cu (36.3). The reference values for all the analyzed metals (except Cd) are higher than those of Dutch system (Cr – 100; Ni – 35; Cu – 36; Pb – 85 and Zn – 140 mg kg^{-1}).

Crnkovic et al. in 2006 have investigated soils of the urban and suburban area of Belgrade, especially concerning their concentrations of potentially toxic metals. The paper is aimed at determining the possible pollution in soils. The total acid soluble concentrations of heavy metals and As in the samples were determined. It was found that they were arranged in the order Zn > Ni > Pb > Cr > Cu > As > Hg > Cd in samples collected in the examined area (the order of the elements is based on their arithmetic mean concentrations). In all the samples collected at 0–10 and 40–50 cm depths from 46 selected sites, the contents of Pb and Zn were lower at the depth 40–50 cm. Using target values given by the Dutch Ministry of Housing, Spatial Planning and Environment, it may be concluded that Belgrade soil can, for the most part, be regarded as unpolluted. Traffic seems to be one of the main sources of these metals, but the influence of other factors cannot be excluded.

Continuous air quality monitoring in the Vojvodina district of Serbia and Montenegro has shown high levels of Cd, especially in the outskirts of large towns such as Kikinda (Vidovic et al., 2005). The construction and metal processing industries located in Kikinda have been emitting significant amounts of heavy metals into the environment. The levels of Cd and Zn were monitored in the region of Kikinda and compared to their concentrations detected in Novi Sad, the capital of Vojvodina, where emission of heavy metals originated mostly from vehicle traffic. The study was performed during the economic sanctions when industrial production decreased drastically. The consequence of that was a significant decrease of the concentration of Cd and Zn in atmospheric deposits. Data collected during 3 years indicated a direct influence of atmospheric deposits on heavy metal distribution in the chain soil-cattle feed-milk. Decreased Cd concentrations of 93% in atmospheric deposits in the Kikinda region resulted in decreased Cd concentrations of 17% in cattle feeds and 13% in milk. Decreased Zn concentrations of 58% in atmospheric deposits caused reduced Zn concentrations of 30% in soil, 17% in cattle feeds, and 17% in milk.

5. Conclusions

According to the studies that have been assessed it can be concluded that there are polluted sites in all Balkan countries related to mining activities, traffic and other causes. Especially, increased concentration of lead in the soil, represent high risk factor for the health of the exposed children population in the city of Veles, Republic of Macedonia. Also, there are hot-spots with heavy metals in other Balkan countries that represent possible adverse health effects to the exposed local population.

6. Recommendations

Even primarily, the existing results show that there is a heavily pollution with heavy metals of the environment in the city of Veles and its surroundings.

This pollution could have strong health impact to the population living in this area. Therefore, it is very important to perform systematically thorough monitoring to determine the anthropogenic impacts to the total environment

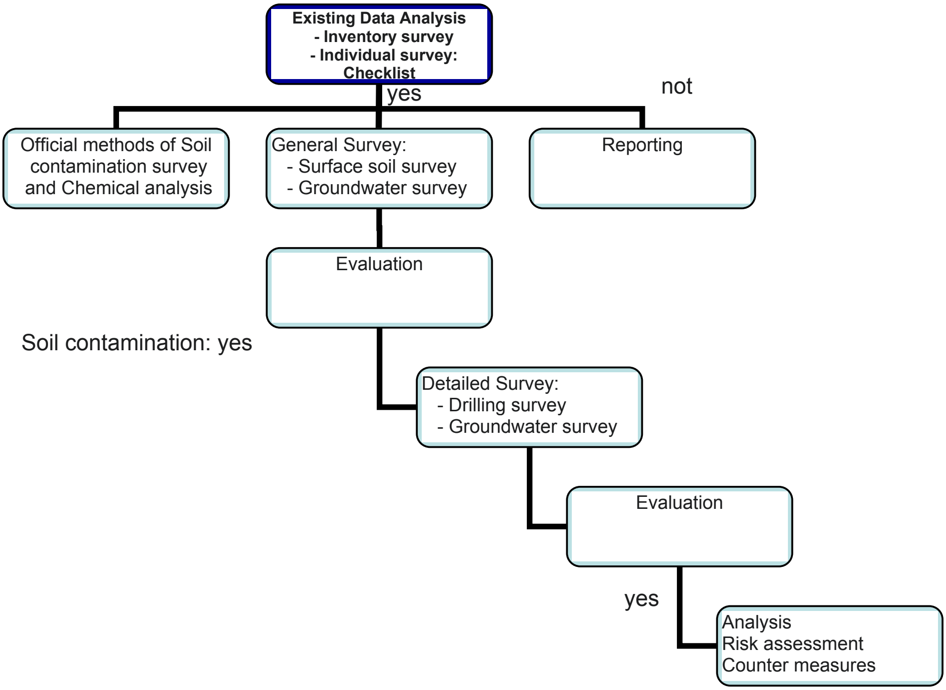

Figure 1. Soil Contamination Survey Method.

(soils, river sediments, air particles, food, and human blood) in the city of Veles, to indicate the temporal evolution of anthropogenic impacts and to determine the bioavailability of heavy metals.

Furthermore, the neuropsychological and psychophysiological investigation in the HERA will give the information of the cognitive impairment in children and adults exposed to the toxic metal environmental pollution in the city of Veles, as well as an assessment of the influence of blood lead level on the central and peripheral nervous system.

From the obtained data the proposing plans for remediation of the polluted sites will be also assumed.

One possible scenario for assessment of the soil contamination could be as following presented on Figure 1.

According to previous studies that have been done it is recommended to establish the Institutional Framework of soil contamination in the Republic of Macedonia regarding its current institutional capacity (Figure 2).

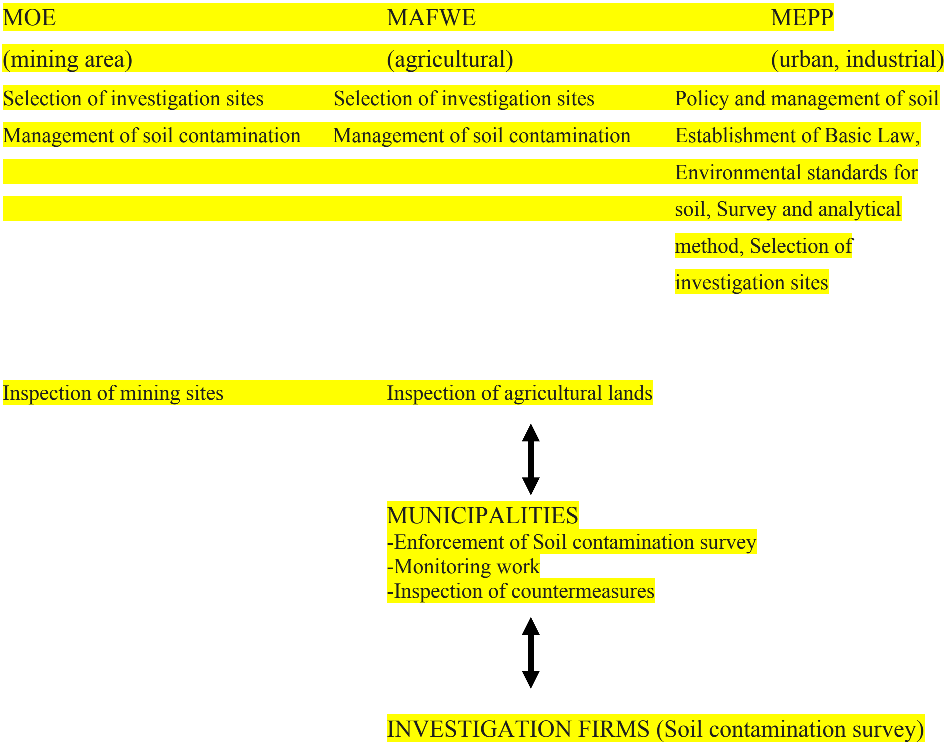

Figure 2. Institutional Framework of Soil Contamination Management.

According to the result of the study performed in the frame of Master Plan were proposed provisional standards for heavy metals in soil in Macedonia (Table 5).

TABLE 5. Provisional standards for heavy metals in soil in Macedonia.

Heavy metal	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
Reference value for soil content value (mg/kg)	25	10	20	200	125	3	70	100	140

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ASSESSMENT OF HEAVY METAL CONTAMINATION IN SOILS AROUND KREMIKOVTSI SMELTER (BULGARIA)

ANNA DIMITROVA[1]*, MOMTCHIL SIDJIMOV[2],
VALERY METODIEV[2] AND ALEXANDER SPASOV[2]
[1] *Department of Materials Science and Technology*
English Language Faculty of Engineering
Technical University of Sofia
8 St. Kliment Ohridski Blvd., 1797 Sofia, Bulgaria
[2] *LABOREX LTD., Laboratory of soil and waste*
15 "Ivan Geshov" Blvd., 1431 Sofia, Bulgaria

Abstract. Heavy metal contamination of soils by metalliferous activities around metallurgical smelter Kremikovtzi was studied in different distances. Concentrations of Pb, Cd, Cu, Zn, Cr, Ni, As and Hg were determined on 38 soil samples (topsoils and subsoils) in order to assess the distribution of these heavy metals in the soil depth. Soils were analyzed using AAS for Pb, Cd, Cu, Zn, Cr and Ni; CVAAS for Hg; and HGAAS for As. Analysis of pH was also defined by pH meter. Correlation between pH values and heavy metal concentrations was not established. Higher metal concentrations were determined in the soil samples taken relatively close to the smelter than those taken at 3 km distance. In some samples determined levels of Pb (330 mg/kg) and As (180 mg/kg) exceed considerably the maximum admissible concentrations (MAS) according to the Bulgarian Legislation (MAS of Pb is 130 mg/kg and MAS of As is 40 mg/kg). Determined concentrations of other metals (Cd, Cu, Zn, Cr, Ni, Hg) are below MAS but exceed the background and precautionary values. Determined levels of heavy metals and arsenic in the surface soil layers and these from 30 cm depth were quantified in the same order of magnitude. It was found that metal concentrations decreased with the distance increasing from the smelter. In addition, soil heavy metal contamination may pose a potential health risk to residents in the vicinity of smelter areas. For that reason, the morbidity rate of population living in this region was analyzed and it was found that allergic, respiratory, cardiovascular and endocrine disorders were with higher frequency rate compared with the average data for the city of Sofia.

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* To whom correspondence should be addressed: a_dimitrova@tu-sofia.bg

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1. Introduction

Metallurgical smelters are one of the most important sources of heavy metals in the environment. Mining, milling and smelting operations provide obvious sources of contamination in the environmental compartments. As a result, elevated levels of heavy metals can be found in and around metalliferous mines and smelters due to discharge and dispersion of gases and wastes into nearby agricultural soils, food crops and stream systems (Dumontet et al., 1992; Barcan and Kovnatsky, 1998). Soil is the main reservoir of large amount of heavy metals and other contaminants. Eventually, metals may pose a potential health risk to residents in the vicinity of smelter areas. Dust from the polluted ground may have toxic effects as a consequence of inhalation or ingestion by humans, particularly children, which poses major health hazards (Folinsbee, 1993; Sanchez-Camazano et al., 1994).

Many studies have been conducted on heavy metal contamination in soils, plants, water and sediments from metalliferous mines and smelters throughout the world. Typically, these studies found highly elevated concentrations of the emitted metals in the topsoils adjacent to the smelting plants. Often, soil metal concentrations were found to decrease with distance increasing from the smelter (Martley et al., 2004; Sichorova et al., 2004).

The aim of this study was to determine the heavy metal concentrations in topsoils and subsoils in different distances in the vicinity of the Kremikovtsi iron smelter and to assess the morbidity rate of the population living in this region.

Kremikovtsi iron smelter is situated in the north-eastern suburbs of Sofia. It occupies 2,357 km^2 on the territory of Sofia hollow and the average sea level in the region is 550 m. It was the biggest ferrous complex in Bulgaria. Its industrial activities began in 1963 and operated until 2009. Metallurgical smelter Kremikovtsi was covered the full metallurgical cycle – from output of iron ore to ready products - cast iron, steel and ferrous alloys. It also produced tin-plated sheet iron and metalloplasts. According to the amounts of production, it was the most important metallurgical complex of Bulgaria. The smelter was identified as an important source of heavy metals in the environment (Faintondjiev et al., 2000a).

2. Materials and Methods

The study area was located around the metallurgical complex. Representative sampling points of soil collection were defined. The sampling locations and topography of the study area are shown in Figure 1. Soil samples were collected at two distances – 1 and 3 km around the smelter in all directions. Sampling was done according to the specifics of rose wind typical for the study area. Soil samples were collected in accordance with ISO 10381-1 and Bulgarian State Standard 17.4.5.01. Each soil sample was collected into two depth intervals, one from the surface soil (0–10 cm) and another in depth 20–30 cm with hang auger. Each soil sample was comprised a composite of 5 subsamples taken from an area of 50 m^2. Sampling collection was realized in spring of 2008 year.

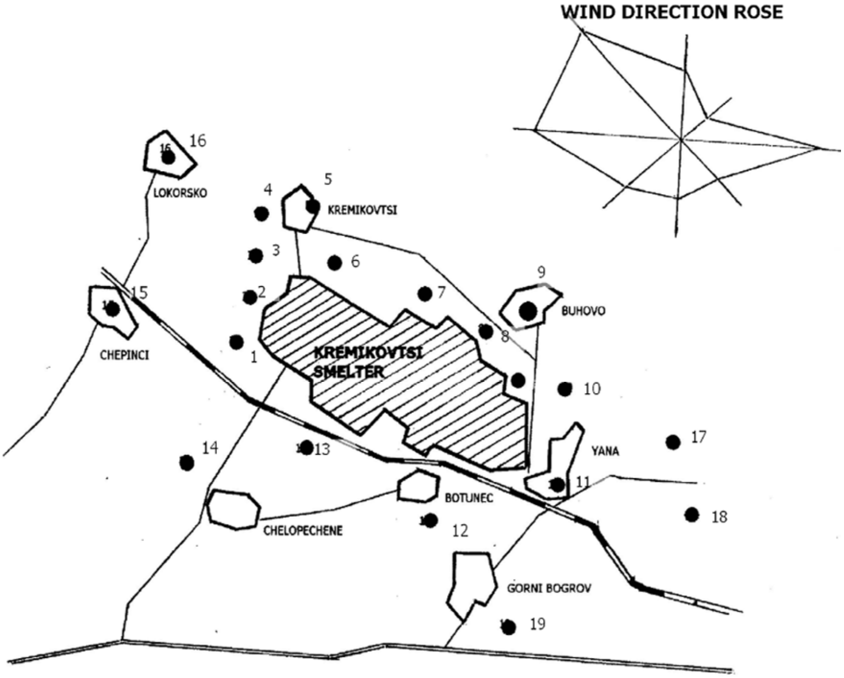

Figure 1. Sampling location map around Kremikovtsi smelter.

The soil pH was determined, according to ISO 10390, by a 5:1 ratio of soil to deionized water, using a pH meter Sartorius PB 11 with glass electrode.

After air-drying at 25°C for 5 days, soil samples were disaggregated, sieved to <2 mm and then ground to a fine powder. The finely milled soils were digested in 3:1 concentrated HCl and HNO_3 (aqua regia). The solutions were analyzed by flammable atomic absorption spectrometry for Pb, Cd, Cu, Zn, Cr and Ni. Concentrations of Hg in samples were measured by cold vapour atomic absorption spectrometry and of As by hydride generation atomic absorption spectrometry.

Accuracy of analyses was checked using international soil standard and duplicate samples. The quality control gave good precision (S.D. < 5%) for all samples.

3. Results and Discussion

The mean and range of all soil pH values are shown in the next tables (Table 1 and Table 2). pH are ranged closely from 6.01 to 8.64 due to the similarity in mineralogy and origin of the soil samples.

TABLE 1. Heavy metals concentration in soil samples taken relatively close to the smelter - about 1 km distance (mg/kg).

Sample	pH	Pb	Cd	Cu	Zn	Cr	Ni	As	Hg
1	6.07	200	0.75	52.0	200	21.0	50.5	76.5	<LOD
1*	6.10	200	1.00	60.0	215	18.5	57.5	75.2	<LOD
2	7.21	82.0	<LOD	42.5	115	27.0	48.0	31.1	<LOD
2*	6.56	82.0	<LOD	45.0	135	27.0	54.5	30.6	<LOD
3	6.75	102	<LOD	42.5	100	22.5	40.0	18.9	0.075
3*	7.26	99.0	<LOD	46.0	100	24.5	44.0	30.6	<LOD
4	8.64	415	1.25	105	330	58.0	34.0	110	<LOD
4*	8.61	315	0.50	139	135	84.0	42.5	140	<LOD
5	7.91	300	1.75	83.0	280	17.5	62.5	175	0.20
5*	7.93	330	1.75	87.5	300	15.5	67.5	180	<LOD
6	7.20	195	0.50	58.0	125	22.5	47.5	51.5	0.18
6*	7.85	200	0.75	60.0	134	27.0	50.5	60.5	<LOD
7	7.39	140	<LOD	33.5	130	20.0	43.0	42.0	<LOD
7*	6.79	155	<LOD	47.0	135	27.0	48.0	50.2	<LOD
8	7.14	81.5	<LOD	38.0	105	21.0	46.5	32.5	0.07
8*	6.68	90.0	<LOD	42.0	118	27.0	54.0	20.5	<LOD
9	6.71	125	<LOD	43.0	125	20.5	35.0	27.0	<LOD
9*	8.15	85.5	<LOD	28.5	65.0	14.5	19.5	30.9	<LOD
10	6.90	102	<LOD	37.5	97.5	21.5	45.0	22.2	<LOD
10*	6.85	111	<LOD	42.0	105	26.0	47.5	25.5	<LOD
11	6.97	125	<LOD	40.0	85.0	20.0	38.0	27.5	<LOD
11*	7.47	110	<LOD	43.5	65.0	25.0	42.5	20.0	<LOD
12	6.53	93.0	<LOD	37.5	90.0	25.5	41.5	16.0	0.06
12*	6.93	90.0	<LOD	35.5	75.0	18.0	40.5	21.6	<LOD
13	8.32	60.0	<LOD	41.0	77.5	21.0	35.5	34.2	0.017
13*	7.52	68.5	<LOD	33.0	77.5	15.5	30.0	33.8	<LOD
MAC		130	2.5	140	390	250	80	40	1.5

x number – samples taken from the surface soil (0–10 cm depth)
x number * – samples taken from 20–30 cm depth
MAS – maximum admissible concentration
Limit of detection (LOD) for Hg is 0.002 mg/kg
Limit of detection (LOD) for Cd is 0.25 mg/kg

TABLE 2. Heavy metals concentration in soil samples taken at 3 km distance (mg/kg).

Sample	pH	Pb	Cd	Cu	Zn	Cr	Ni	As	Hg
14	8.20	87.5	0.25	47.5	115	18.0	24.0	30.0	0.058
14*	7.13	80.0	0.25	38.0	120	14.0	32.5	17.5	<LOD
15	6.43	225	1.00	52.5	255	18.0	50.5	93.0	0.051
15*	6.99	180	0.75	38.5	270	15.0	30.5	56.5	0.028
16	6.3	135	0.50	28.0	120	9.0	22.0	38.5	<LOD
16*	8.13	142	0.75	32.0	128	15.0	28.0	43.0	<LOD
17	6.73	46.5	<LOD	25.0	90.0	10.5	14.5	5.10	<LOD
17*	6.84	29.0	0.25	52.0	115	13.5	19.0	11.0	<LOD
18	6.30	81.5	0.25	45.0	110	18.5	34.5	45.0	0.060
18*	6.01	72.0	<LOD	38.0	95.0	21.0	29.5	29.5	<LOD
19	6.43	66.0	<LOD	32.0	68.0	17.5	28.5	39.5	<LOD
19*	6.99	64.5	<LOD	27.5	95.0	16.0	21.5	40.0	<LOD
MAC		130	2.5	140	390	250	80	40	1.5

x number – samples taken from the surface soil (0–10 cm depth)
x number * – samples taken from 20–30 cm depth
MAS – maximum admissible concentration
Limit of detection (LOD) for Hg is 0.002 mg/kg
Limit of detection (LOD) for Cd is 0.25 mg/kg

The concentrations of heavy metals in soils taken relatively close to the smelter (1 km distance) are given in Table 1 and of soil samples taken at 3 km distance in Table 2. Because of its anomalously high metal content, sample 4 and 4* were considered as outliers of the total dataset.

Results from atomic absorption analysis were shown high concentrations of heavy metals and arsenic in the soils. In some samples (samples 1, 1*, 5, 5*, 6, 6*, 7, 7*, 14, 14*, 16, 16*) determined levels of Pb and As considerably exceed the maximum admissible concentrations (MAS) according to the Bulgarian Legislation. These higher concentrations were measured in samples collected from the wind direction. Dust emissions from the iron smelter were suspected as the main source of this pollution. As no air filtering devices had been installed in the smelter, dusts emitted from the Kremikovtsi smelter had been drifted by wind as far as Sofia about 20 km west of the smelter. Determined concentrations of other metals (Cd, Cu, Zn, Cr, Ni, Hg) are below MAS but exceed the background and precautionary levels as set by the ordinance. These levels of heavy metals are probably caused by the smelter emission and atmospheric deposition which can lead to serious soil contamination in the surrounding vicinity. Based on the present study, it had been hypothesized that the metal smelter was the cause of wide-spread heavy metal pollution of the soils in the surrounding communities.

Content of heavy metals and arsenic in the surface soil layers and those from 30 cm depth were quantified in the same order of magnitude. The results from previous similar studies were shown that heavy metals and arsenic in soil surface layer were higher compared with their content in 20–30 cm soil depth (Spasov et al., 1996). Established similarity of metal concentration in soil depth may be based on mechanical treatments of agricultural soils.

It was found elevated metal concentrations in the soils adjacent to the Kremikovtsi complex that were decreased within 3 km distance from the boundary of the industrial area to concentrations considered as background levels. However, this study was based on only 38 soil samples, and the decrease of the soil concentrations with distance increase from the smelter was not clearly expressed.

Measured heavy metal concentrations were not correlated with pH. Owing to the narrow range of pH measured in the samples, this parameter has limited importance on the heavy metal distribution, substantially limiting their mobility because of the neutral-subalkaline environment.

Up to now many studies were conducted to determine the soil contamination with heavy metals around metallurgical smelter Kremikovtsi (Spasov et al., 1996; Balabanov, 1999; Faitondjiev, et al., 2000 a,b). Determined levels of heavy metals in soils found in this study were higher than those established in earlier studies (average values of Pb – 66.8 mg/kg, Cd – 0.35 mg/kg, Cu – 36 mg/kg, Zn – 50 mg/kg and As 17.5 mg/kg (Spasov et al., 1996).

Compared to average concentrations in industrial and urban soils in the world (Table 3), the median values of Pb in the analyzed soils were much lower than those reported of samples from some large and/or industrialized cities (i.e. Boston, central Madrid, central London), but they were similar to those measured in smaller cities (i.e. Aberdeen, Madrid). Cu, Cr, Cd and Zn concentrations were generally similar to those reported for other cities, while Ni contents were generally higher. Records for Hg levels in urban soils are scarce in the literature.

TABLE 3. Average heavy metal concentrations (mg/kg) in soil from different cities in the world.

City	Pb	Zn	Cu	Cd	Cr	Co	Ni	Reference
Hamburg	218	516	146	2	95.4	–	62.5	Lux (1986)
Madrid	161	210	71.7	–	74.7	6.42	14.1	De Miguel et al. (1998)
Aberdeen	94.4	58.4	27	–	23.9	6.4	14.9	Paterson et al. (1996)
Central London	647							Rundle and Duggan (1980)
Boston	800							Spittler and Feder (1979)
Central Madrid	621							Pellicer (1985)

4. Health Status of the Population from Kremikovtsi Region

In general, the health status of the population is determined by many factors of the environment and working places, social welfare and hereditary factors. In this case to the region of Kremikovtsi there are additional resources for industrial pollution with possible impact on health indicator levels in the region.

It was examined record morbidity data for 2 years – 2007 and 2008. In Tables 4 and 5 were considered diseases of adult and children population, according to the requirement of International Classification of Diseases-10 for 16th Diagnostic Consultation Center which serves a large contingent of residents in Kremikovtsi region- from built-up areas: Botunec, Yana, Buhovo, Gorni Bogrov, Dolni Bogrov, Lokorsko, Kremikovtsi, Chepinci, Chelopechene.

According to the exposed data it can be inferred that the health status of Kremikovtsi population is characterized by levels of morbidity indicators similar to those for the Sofia city population. There are some exceptions of the morbidity levels of respiratory diseases and indications, symptoms and abnormal of XVIII Class. They are significantly higher than levels of children from Sofia. Increased morbidity levels of the cardiovascular, respiratory and endocrine disorders are determined on adults.

TABLE 4. Adults Morbidity – registered data in 16th DCC.

Registered morbidity ICD-10	General number2 007	Relative part	General number 2008	Relative part	Sofia 2008	
					Number	Relative part
Total number of diseases	13,537	100	10,412	100	2116,182	100
Infectious diseases and parasitosis/ A00-V99/-class I	0	0	0	0	11,877	0.56
Neoplasm /C00-D48/-class II	33	0.24	29	0.28	56,812	2.68
Diseases of the blood, hemorrhage and certain disorders involving the immune mechanism /D50-D89/-class III	18	0.13	39	0.37	13,528	0.64
Endocrine diseases, disorders of nutrition and metabolism /E00-E90 -class IV	601	4.44	0	0.00	174,620	8.25
Mental and behavioral disorder /F00 -F99/-class V	0	0	752	7.22	43,581	2.06
Nervous system diseases / G00-G99/ -class VI	952	7.03	958	9.20	139,274	6.58
Diseases of the eye and its appendages/ H00-H59/-class VII	3,353	24.77	2,373	22.79	211,491	9.99
Ear Diseases /H60-H95/-class VIII	634	4.68	427	4.10	93,827	4.43

(*continued*)

(*continued TABLE 4.*)

Registered morbidity ICD-10	General number2 007	Relative part	General number 2008	Relative part	Sofia 2008	
					Number	Relative part
Diseases of the blood circulatory system /I00-I99/-class IX	1,851	13.67	2,325	22.33	440,727	20.83
Respiratory diseases/J00-J99/-class X	1,336	9.87	902	8.66	259,477	12.26
Diseases of the digestive system/K00-K93/class XI	1,375	10.16	439	4.22	92,593	4.38
Diseases of skin and subcutaneous tissue/LOO-L99/-cass XII	210	1.55	251	2.41	80,891	3.82
Diseases of the musculoskeletal system and connective tissue/M00-M99/- class XIII	1,677	12.39	578	5.55	145,022	6.85
Diseases of Genitourinary System/N00-N99/-class XIV	983	7.26	956	9.18	235,672	11.14
Pregnancy, birth and perinatal period/O00-O99/-class XV	27	0.20	0	0	15,711	0.74
Some states arising in perinatal period/P00-P96/-class XVI					0	0.00
Congenital anomalies/defects of the development/deformations and chromosomal aberration /Q00-Q99/-class XVII	0	0	0	0	2,190	0.10
Symptoms, indications and abnormal found in clinical and laboratory tests unclassified in the other groups /R00-R99/-class XVIII	125	0.92	0	0	23,925	1.13
Injuries, poisoning and certain other consequences of external impact influence /S00-T98/-class XIX	362	2.67	383	3.68	74,964	3.54

TABLE 5. Children Morbidity– registered data in 16th DCC.

Registered morbidity ICD-10	General number 2007	Relative part	General number 2008	Relative part	Sofia 2008	
					Number	Relative part
Total number of diseases	4,872	100	4,428	100	289,490	100
Infectious diseases and parasitosis/ A00-V99/-class I	0	0	0	0	10,100	3.49
Neoplasm/C00-D48/-class II	0	0	0	0	778	0.27

Diseases of the blood, hemorrhage and certain disorders involving the immune mechanism/D50-D89/ -class III	17	0.35	12	0.27	2,200	0.76
Endocrine diseases, disorders of nutrition and metabolism/E00-E90/ -class IV	0	0	7	0.16	6,173	2.13
Mental and behavioral disorder/ F00-F99/-class V	0	0	0	0	4,215	1.46
Nervous system diseases/G00 -G99/class VI	1	0.021	0	0	7,142	2.47
Diseases of the eye and its appendages /H00-H59/-class VII	0	0	0	0	25,650	8.86
Ear Diseases/H60-H95/-class VIII	0	0	0	0	22,295	7.70
Diseases of the blood circulatory system /I00-I99/-class IX	0	0	0	0	2,688	0.93
Respiratory diseases/J00-J99/ -class X	4,446	91	3,905	88.2	137,188	47.39
Diseases of the digestive system/ K00-K93/-class XI	18	0.37	32	0.72	9,933	3.43
Diseases of skin and subcutaneous tissue/- LOO-L99/- class XII	24	0.49	109	2.46	13,963	4.82
Diseases of the musculoskeletal system and connective tissue/M00/M00-M99/-class XIII	4	0.082	2	0.045	6,000	2.07
Diseases of Genitourinary System/N00-N99/-class XIV	11	0.23	11	0.25	14,366	4.96
Pregnancy, birth and perinatal period/O00-O99/-class XV	0	0	0	0	352	0.12
Some states arising in perinatal period/ P00-P96/-class XVI	0	0	0	0	1,726	0.60
Congenital anomalies/defects of the development and chromosomal aberration/Q00-Q99/-class XVII	0	0	11	0.25	3,113	1.08
Symptoms, indications and abnormal found in clinical and laboratory tests unclassified in the other groups /R00-R99/-class XVIII	351	7.20	339	7.66	10,949	3.78
Injuries, poisoning and certain other consequences of external impact influence/S00-T98/class XIX	0	0	0	0	10,659	3.68

5. Conclusions

The data obtained in this study demonstrated high concentrations of heavy metals and arsenic in soils around metallurgical smelter Kremikovtsi. Determined levels of metals (Cd, Cu, Zn, Cr, Ni, Hg) were below the maximum admissible concentrations but exceed the background and precautionary values. And in some soil samples measured concentrations of lead and arsenic considerably exceed MAS according to the Bulgarian Legislation.

In comparison with world data from industrial areas concentrations of Pb were much lower, these of Cu, Cr, Cd and Zn were similar and Ni content was generally higher. But present results were higher compared to previous data of soil pollution in Kremikovtsi region.

It was found that determined metals content in the surface soil and these from 30 cm depth were in the same order of magnitude. And metal concentrations decreased with the distance from the smelter.

In addition, the morbidity rate of groups of the population (children and adults) living in this region was analyzed and it was found that respiratory, allergic, cardiovascular and endocrine disturbances to be with higher frequency rate compared with the average data for the city of Sofia.

The results from this study demonstrated harmful effects in the environment and human health. Regular ecological monitoring would be advisable in the next few years for Kremikovtsi region with a goal to attend metal concentrations changing and to prevent adverse health effects of vulnerable group of population.

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COMPARISON STUDY OF PHYSICOCHEMICAL PARAMETERS EVALUATION BETWEEN DANUBE RIVER DELTA AND AXIOS- VARDAR RIVER VALLEY (2003–2009)

FOKION VOSNIAKOS[1]*, GABRIELA VASILE[5],
TRIANTAFYLLOS ALBANIS[2], JANA PETRE[5],
ELENA STANESCU[5], LILIANA CRUCERU[5],
MARGARETA NICOLAU[5], MIHAIL KOCHUBOVSKI[4],
DRAGAN GJORGJEV[4], KONSTANTINOS NIKOLAOU[3],
MARIANA GOLUMBEANU[6],
KONSTANTINOS VOSNIAKOS[1]
AND GEORGE VASILIKIOTIS[1]
[1] *Balkan Environmental Association (B.EN.A.),*
Alexander Technological Education Institute of Thessaloniki
P.O. Box 141, 57400-Sindos, Thessaloniki, Greece
[2] *Department of Chemistry, University of Ioannina*
Panepistimioupoli Ioannina, Greece
[3] *OMPEPT - Organisation for the Master Plan and*
Environmental Protection of Thessaloniki, Greece
[4] *Institute of Public Health of the FY Republic of Macedonia*
50 Divizija No. 6, 1000 Skopje, FY Republic of Macedonia
[5] *National Research and Development Institute for*
Industrial Ecology (ECOIND)
Panduri 90-92, Bucharest, Romania
[6] *International Research Secretariat of B.EN.A.*
National Institute of Marine Research and
Development(NIMRD) "Grigore Antipa", 300 Mamaia Bd.,
900581 Constanta, Romania

Abstract. The main objective of this paper is to provide data on the Danube River Delta Biosphere (Romania) aquatic ecosystem quality and Vardar – Axios River Valley (FYR Macedonia – Greece) on comparison basis from on the long-term analytical investigation for specific indicators. In particular this paper is focused on the quality assessment of surface water, sediment and biota of the

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* To whom correspondence should be addressed: bena@gen.teithe.gr

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Danube Delta Biosphere Reservation. Information is presented on water and sediment quality variations in heavy metals over a seven-year period (2003 and 2009), from April to October. Samples have been collected monthly from two different locations (Murghiol and Uzlina) situated on St. Gheorghe branch. The presence of some toxic metals was noticed in surface waters and sediments. In both locations in different periods of time high concentrations of Fe, Mn, heptachlor, Lindane, PCBs, phenols, mineral oil in surface water represent an acute problem for the inhabitants of the Danube Delta which use water for household consumption directly from the Danube, without any treatment. This presents a series of disadvantages, such as a high risk of disease for the population, mostly for children and old people. The evolution of the pollution levels of Danube waters was compared with that of Axios/Vardar River, another major transboundary river in the Balkan area.

Keywords: Danube Delta, Axios – Vardar River, ecosystem, metals, pollution, drinking water, health, sediment, benthic macro invertebrates

DANUBE DELTA BIOSPHERE

1. Introduction

The Danube, the second largest river in Europe after Volga, flows to several countries from where it receives discharges of agricultural, industrial and urban effluents. The Danube River waters flow into the Black Sea through its delta (5,600 km^2), which constitutes the largest wetland from Europe. Designated by UNESCO as a Ramsar World Heritage and a reservation of the biosphere, the Danube Delta is the third delta in ecological significance among the 300 reserves of the world. The delta wetlands have many important functions contributing to the ecological, social and economical wealth of the region. The most important threats to the Danube Delta are represented by changes in the quantity and quality of water reaching from upstream catchments.

Danube Delta Biosphere Reservation (DDBR) includes Danube Delta, the complex Lagoon Razim-Sinoe, navigable Danube up to Cotul Pisicii, the district Isaccea –Tulcea including floodable zone, the area from Lake Saraturi - Murighiol to Plopu and finally the Black Seaside from Chilia Branch to Midia Cape. On the reservation territory live about 14,000 inhabitants, according to 2005 statistics (Romanian Government, 2009).

There are three categories of areas in DDBR: first is the core area – strictly protected area, were no humans activities are allowed, except research. The second category is the buffer area, were traditional activities are allowed and the third category is the economic area were economic activities are allowed, including commercial fishing (Tudor et al., 2006).

Disturbed aquatic ecosystems, biodiversity and habitats are occurring in the Danube Delta due to a large number of activities, including navigation, intensive agriculture, fish farming, and most recently recreation and tourist industry. In many areas of the Danube Delta, the combination of these practices had led to a decrease of the river's self-cleaning capacity and to an increase of polluted effluents resulting in a continuous deterioration of river quality (Vosniakos et al., 2006; Vosniakos et al., 2008; Vosniakos et al., 2010; Gils et al., 2005). Such activities often conduct to supplementary quantities of nutrients and potentially hazardous substances as heavy metals, mineral oil, persistent organic compounds including pesticides and PCBs, into the Danube Delta ecosystems.

One of the acute problems of the inhabitants of the Danube Delta is the lack of drinking water, wastewater networks and treatment plants (Romanian Government, 2009). Using water directly from the Danube for household consumption, while the water quality has deteriorated greatly over the reference period 1950–1960, presents a series of disadvantages, such as: a high risk of disease for the population and impossibility to promote economical activities, which requires drinking water. Also, the local population can not develop and practice some tourism activities, which would represent an important source of income.

The lack of sewerage system and wastewater treatment plants involves uncontrolled discharges of wastewaters with various loads of pollutants, which affect both human health and the quality of the environment, generate pollution and leading the degradation of the environmental factors. As is recording in research study, water pollution in the DDBR has contributed to losses of biodiversity (Cioaca et al., 2009). Therefore, a great concern is focused on water quality.

Contamination by potentially toxic metallic elements in the natural environment is one of the major problems for human health and environmental quality because these elements are indestructible and most of them have toxic effects on living organisms when exceed a certain concentration (Ghrefat and Yusuf, 2006). Trace elements may be introduced into the aquatic systems by both natural processes (weathering and corrosion) and because of human activities (heavy nutrients loads from agricultural fertilizers and heavy metals from industry) (Teodorof et al., 2007; Sakan et al., 2009). Sediment represents one of the ultimate sinks for heavy metals discharged into the aquatic environment. Contaminants are not necessarily fixed permanently by the sediments, and under changing environmental conditions metals may be released to the water column by various processes of remobilization. Thus, in aquatic ecosystems, sediments may be both a carrier and possible source of pollution (Forstner, 1989).

The total concentration of metals does not provide suitable information on the bioavailability of hazardous elements and the speciation study is a necessary tool to obtain this information (Tuzen, 2003; Vasile and Tanase, 2008). Many leaching tests, often-single procedures, have been developed and implemented to evaluate the reactivity of metals in sediments. For sediments and suspended particles, the use of selective leaching methods can provide key information on the nature of metal species bound to the solid phase such as: exchangeable ions; carbonates, with some iron and manganese oxides; readily reducible species (certain oxides); oxidisable organics and sulphides (Ure and Davidson, 2002). As the final step, a total digestion with a mixture of strong acids could provide the lithogenous fraction (Gagnon et al., 2009).

The mobility, transport and partitioning of trace metals in aquatic systems is a function of the chemical forms in which they appear or can be transformed into. Heavy metals of anthropogenic origin are generally introduced in the environment as inorganic complexes or hydrated ions that are easily adsorbed on sediment particles surface through relatively weak physical and chemical bonds. Thus, these elements are found predominately as labile and easily extractable fractions in sediment. On the contrary, the products of weathering of clay minerals, which are generally very rich in heavy metals, contain these elements in less available forms (Pempkowiase et al., 1999). Benthic organisms are generally in contact with sediment and can ingest sediment particles; thus, the dietary vector can modulate the biological metal uptake in contaminant-impacted receiving zone. The metal accumulation in aquatic organisms often depends upon the capacity of sediment to retain metals (Kersten, 2002).

The phytoplankton community represents the bulk that brings into the ecosystems the energy, determines existing food chains in an aquatic ecosystem and constitutes the trophic structure of many aquatic ecosystems. These are represented by microscopic unicellular algae, colonial or filamentous mass flowing water and due to the short life cycles, respond quickly to changes in the aquatic environment. The zooplankton organisms are particularly sensitive to pollution, so many of these bodies are used as bioindicators it's consisting of various level consumers which populate the entire mass of water. The zooplankton is very well represented by the betamesosaprobic rotifers, betamesosaprobic species of the cladocers and oligo-betamesosaprobic copepoda. Aquatic benthonic community consists of integrated population biocenosis that lives freely or inside of own houses on the water bottom or attached to rocks or other submerged objects.

The monitoring of the specific components of the benthonic biocenosis plays an important role in establishing the water quality for the determination of the pollution risk, because the benthonic species respond in the most realistic way to the environmental changes. The zoobenthos density and diversity are dependent on the bottom sediment quality, the condition of running, the presence and/or absence of pollution sources (Beasley and Kneale, 2004).

To reduce the negative effects of pollution, it is necessary to understand how pollutants are transmitted from the water to the biotic components (flora and fauna species), due to interdependency relations.

In 2005, the Romanian Ministry of Environment approved a Master Plan for sustainable development of DDBR. The most important issues were improvement of monitoring system for the Danube Delta ecosystems using modern surveillance; improving of infrastructure, utilities, transport and communications; support for the development of alternative economic activities in order to reduce pressure on fisheries resources.

According to Government Decision 1516/2009 (Romanian Government, 2009) until 2013 in all communities situated in DDBR the drinking water networks supply will be introduced. In the same time sewage networks and wastewater treatment plants will ensure the quality of water discharged into the Danube Delta.

Protection and conservation of this Biosphere Reserve ecosystem need a long-term assessment of the physico-chemical and biological water quality in order to provide information concerning the discharge of different pollutants, and to point out the potential trend of eutrophication.

In the period of spring to autumn of the years 2003–2009 samples of surface water, sediments and biota from to Uzlina and Murighiol Locations, situated in Danube Delta on Saint Gheorghe Branch, were collected. The main objectives were the identification and quantification of the most important inorganic and organic pollutants in water and sediment at both locations, the evaluation of their compliance with standards, and the long-term trends of the ecosystem's chemical status.

The Saint Gheorghe branch is the most southern branch carrying 23% of the Danube water discharge and 21% of the Danube sediment discharge (Jugaru et al., 2009). This highly sinuous branch was cut off in 1984–1988 by an artificial canal opened to shipping, which caused significant changes of the river flow velocity in the natural channel. The artificial canal that has also an increased velocity captured the main flow (82%). In Uzlina (45°4′N–29°14′E) situated on the natural channel, samples were collected from the Cormoran Complex, a place with intensive naval activity. Cormoran Complex has its own domestic wastewater treatment station, and the treated water is dumped into the river. However, many houses in this area are discharging water directly into the river.

In Murghiol (45°2′N–29°10′E), samples were collected from the artificial canal near Murghiol Lake. These two locations situated on two different channels, in an area of natural fish reproduction and a pelican colony, are tourist villages in continuous expansion with ports and fuel stations for boats (see the map of the Danube Delta on Figure 1).

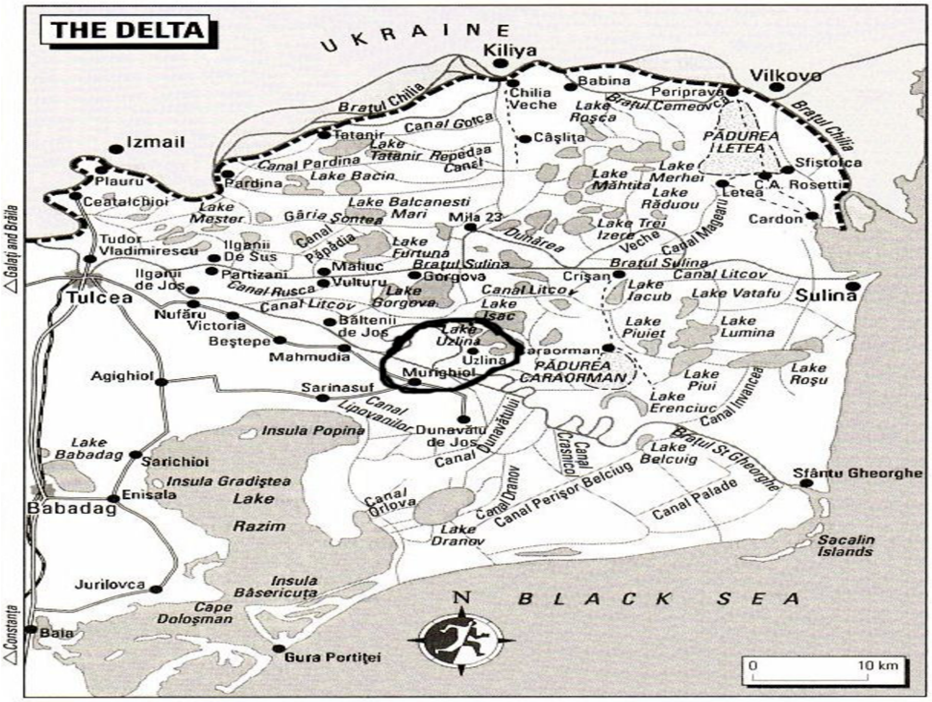

Figure 1. The map of Danube Delta: Uzlina and Murighiol Locations on Saint Gheorghe Branch.

2. Materials and Methods

2.1. SAMPLE COLLECTION

Sampling of surface water, sediment and biota (phytoplankton and zooplankton from surface water and benthic macroinvertebrates from sediments) was performed monthly in the period April to October (2003–2009) from Uzlina and Murighiol Locations. In April 2005 and 2006, sampling from these two locations was not possible because of floods with a very high level of water-flow. The sampling and preservation step was done according to the recommendations of specific international guides (ISO 5667/ parts 1, 3, 6, 12; EN ISO 9391).

Water samples were collected and stored in glass bottles. From each sampling location, 10 l of water were collected. The sediment samples were taken from 2 to 3 m within the Danube using a Van Veen Bottom Sampler. All samples were kept in cooling boxes at 4°C during transportation and the analyses were performed immediately after receiving the samples in the laboratory.

2.2. POLLUTANTS AND QUALITY INDICATORS

In all the samples (surface water and sediments) were performed analytical determinations of toxic metallic elements such as arsenic, chromium, cadmium, copper, nickel, lead, mercury, iron and, zinc.

A study of metallic elements mobility from sediment samples was accomplished in order to determinate the influence of harmful metals to alive organisms from water body.

2.3. REFERENCE COMPARISON VALUES

The Romanian Norm 161/2006, transposed from Directive 2000/60/EC introduced a classification of the quality factors that describe the ecological condition of surface water. This classification consists in five conditions for surface water: *very good* (I), *good* (II), *moderate* (III), *poor* (IV) and *bad* (V). The parameters evaluated for the chemical and physico-chemical characterization of water quality are: temperature, oxygen, nutrients, pollution with dangerous substances, pH and the mineralization (Ca, Mg, Na). The Norm provides two categories of chemical conditions for all aquatic, sediment and biota systems: good chemical conditions (all quality parameters for the indicators are situated in the limits imposed by the quality standards) and bad chemical conditions (one or more parameters exceed the limits imposed by the quality standards).

2.4. METHODS AND EQUIPMENTS

Analytical techniques used for determination of metallic elements from water and sediment samples (total and mobile fractions) were flame AAS performed on THERMO SCIENTIFIC M6 DUAL SOLAAR equipment; cold-vapor technique coupled to AAS using an ATI UNICAM SOLAAR 929 Spectrometer, for Hg analysis; ICP-EOS technique with a PERKIN ELMER OPTIMA 5300 DV equipment for As concentrations.

- Four different analytical methods using single chemical extraction (chemical reagents with different aggressiveness) were applied to the sediments collected from Uzlina and Murighiol locations in order to establish the *sediment structure*.
  - First method was used for extraction of mobile metallic elements. The working procedure was as follows: 4 g of wet sediment (particle size <60 μm) were mixed with 40 mL of buffer solution of 1M ammonium acetate and 0.01 M EDTA (pH = 7.2 ± 0.2). The obtained suspension was shaked at 20ºC for two hours with 40 rot/min. The solutions were immediately filtered through a filter paper with porosity of 0.45 μm, rinsed with extraction solution, followed by distilled water and then analyzed using AAS methods (Vasile et al., 2005, 2008; Vasile and Tanase, 2010).
  - The second method was used to extract the metallic fraction bouded by the organic matter and managanese oxides. The extraction solution was a buffer mixture (pH = 4.60 ± 0.05) of 0.025 M EDTA, 1 M CH_3COONH_4 and 0.1 M $NH_2OH.HCl$. The report between sediment and solution was 1:10 and the obtained suspension was shaked 2 h at 40 rot/min and 20°C. The fractions extracted with this method include both the mobile metallic fraction and the metallic fraction bouded by the organic matter and managanese oxides (Liebe, 1999; Vasile et al., 2005).
  - The third single chemical extraction was performed with 1M HCl, at the report between sediment and solution at 1:10, 10 min at 40 rot/min and 20°C and were extracted the metallic fractions bounded by poorly crystalline iron – oxides and organic matter and also the preceding fractions (McCredy et al., 2003; Vasile et al., 2005).
  - The last method, which has the highest aggressiveness, used aqua regia mixture ($HCl:HNO_3$ = 1:3), 2 h on a hot plate (sand bath) and extract the metallic fraction bounded by strong crystalline iron – oxides fraction, residual fraction and also the preceding fractions (ISO 11047, 1998). This method was performed for total metal contents.
- A study for evaluation of the mobile metallic fraction in wet/dried sediment samples was performed with the first method described above. Each sample was divided in two sub-samples, one was dried on air condition and the other one was sieved in a Fritsch Analysette, 3 Spartan Vibratory Sieve Shaker, using a sieve of 63 μm. In case of wet sediments, a dry matter correction (using the percentage of the humidity obtained on a separate sub-sample) was performed before the suspensions were prepared, in order to maintain same ratio between sediment and solution (1:10). The single chemical extraction procedures were performed

three times on each sediment sample, the result representing the average. In addition, blank extraction (i.e. without sediment) was carried out for each set of analysis, using the same reagents.

- *Mobile inorganic species of metallic Hg* were extracted from wet sediment samples with an acid solution (pH = 5) of 1 M $CaCl_2$, in specific conditions: 20ºC, 2 h at 40 rot/min and a report between solid and extraction solution at 1:10 (w/v). Tin chloride was used as a reducing agent. (Han et al., 2003; Martinez et al., 2006; Neculita et al., 2005; Zagur et al., 2005).
- *Mobile inorganic and organic species of metallic Hg* were extracted from wet sediment samples with an extraction solution of 5% HCl and 10% C_2H_5OH at pH situated in the range 1–3. Four times a quantity of 1.5 ± 0.5 g of wet sedimend was extracted with 2.5 mL of solution, 7 min in an ultrasonic bath at 60 ± 2ºC. After each extraction, the obtained solution was collected and the 50 mL volumetric flask was completed with 0.5 HNO_3. Sodium borohydride was used as a reducing agent (Han et al., 2003). The analysis was performed instantly in order to preserve the mobile Hg.
- The sediment dry weight was determined on a separate sub-sample: approximately 1 g was dried to 105 ± 2ºC, up to constant weight. A correction to "dry matter" (quantity of metal per 1 g of dry sediment) was applied to all analytical results.
- The analysis of the *biotic communities* in both sampling sites were focused on the quantitative (numerical density, biomass, abundance after numerical density and biomass) and qualitative components (dominant species, indicator species). Density was estimated with two methods using an inverted microscope. To calculate biomass (bio-volume), the species were approximated as simple geometric or combined forms (Rott, 1981; Edler, 1979). Species richness was referred to the number of algal registered in each sample in the Kolwitz counting chambers (Zalocar de Domitrovic, 2003) using Motic digital microscope.
- In order to quantify the *benthonic macro invertebrates*, the preserved samples were washed under running water through a sieve of 0.50 µm and then sorted by groups of indicators and analysed using Motic stereomicroscope with digital camera. The qualitative species were determinate using Süβwasserflora von Mitteleuropa key (Godeanu, 2002).

2.5. QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC) DATA

The samples were analysed in Department of Pollution Control, which is accredited by RENAR (Romanian Accreditation Association) and follows the requirements of ISO 17025/2005 standard. The Department has certification with BVQI (Bureau Veritas Quality International) in accordance with ISO 9001 standard and has periodical participation to internal and external audits.

For all the analyzed parameters, the laboratories uses standard methods (ISO, SR ISO, EN, SR EN, SR, STAS, EPA), reference materials and certified reference materials. All these standard methods are verified and the main performance parameters (limit of detection, limit of quantification, linearity, accuracy, precisison, selectivity, the uncertainty of measurement) are established with the existing equipment from the laboratories.

The department participates every year at tests for the evaluation of its capability by interlaboratories comparisons (IMEP Belgium, IAWD Germany, CALITAX Spain, Ielab Spain, Quality Infrastructure Danemark) for different groups of pollutants (organic compounds, metallic elements, inorganic compounds) from complex matrixes (surface water, waste water, drinking water, soil, sediment, sludge).

3. Experimental Data

3.1. SURFACE WATER

The metal load grade of the water-body has been generally low. In Uzlina Location were recorded usually iron concentrations higher than the limit value for class II surface water quality according to Romanian Norm 161/2005. In Murighiol Location were recorded high concentrations of iron and manganese in all investigated periods.

In Uzlina Location, the highest value of iron content, six times higher than the class II limit, was recorded in July 2003. According to the data presented in Figure 2, in most cases the detected concentrations of iron were situated over the admissible value for surface water quality, class II (2003, 2004; July–October 2005 and 2006; April, June, July, October of 2007). As shown in Figure 3, in both channels high iron concentrations occur in the same months indicating anthropogenic contamination of the river before the cutoff of the St. George branch. The observed variation of heavy metals in the river water is expected to be caused by influx of contaminants into the Danube through the tributaries polluted by mining and ore treatment activities. In 2008 and 2009, a general decrease in metal concentrations was observed. The values were situated below the limits set for a good water quality status.

In Murighiol Location were recorded high concentrations of iron during the entire period of time. The highest Fe and Mn values were found in 2003 (1.27–8.07 mg/L Fe; 0.63 mg/L Mn) (Figures 3 and 4). High levels of iron, chromium, manganese and nickel were measured occasionally in 2007. The highest concentrations of these metals were recorded in June: 70 µg/L for Cr, 160 µg/L for Mn, 3.72 mg/L for Fe and 460 µg/L for Ni. High values for Ni, specific for a bad chemical status of the water stream, occurred also in the autumn months (180 µg/L in September and 140 µg/L in October).

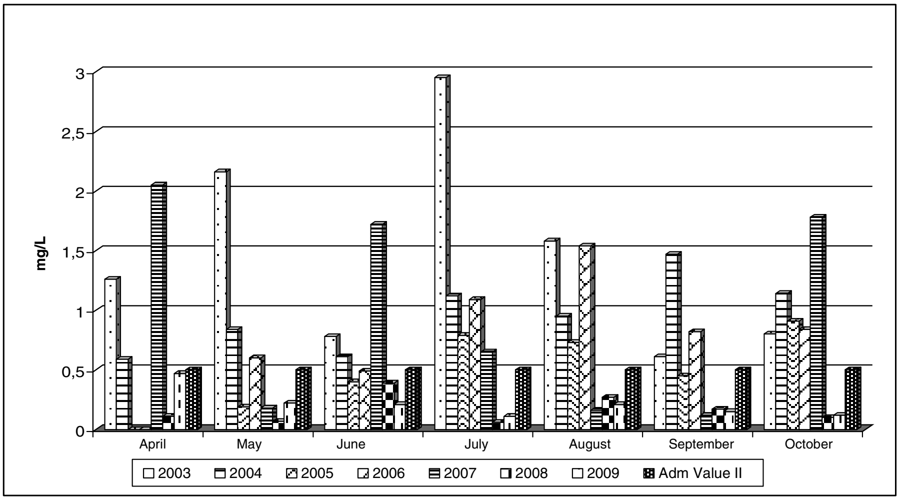

Figure 2. The Iron concentrations in surface waters collected from Uzlina Location.

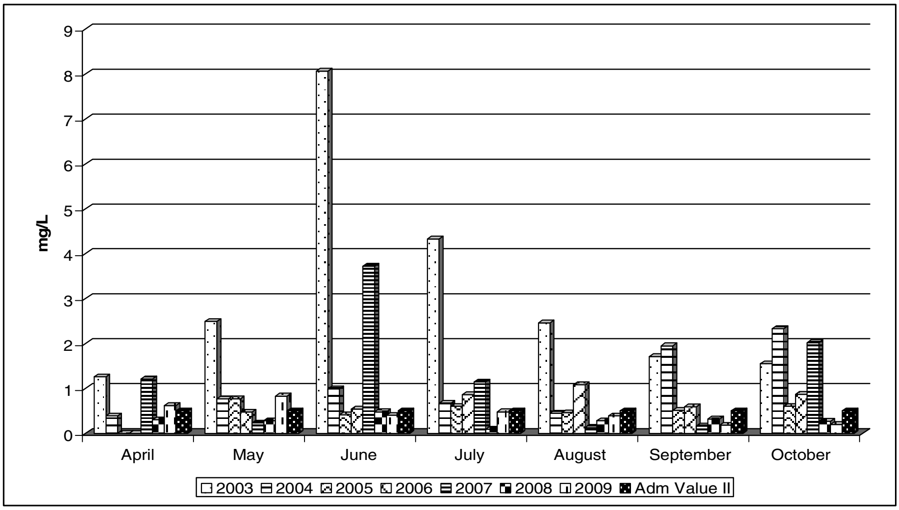

Figure 3. The Iron concentrations in surface waters collected from Murighiol Location.

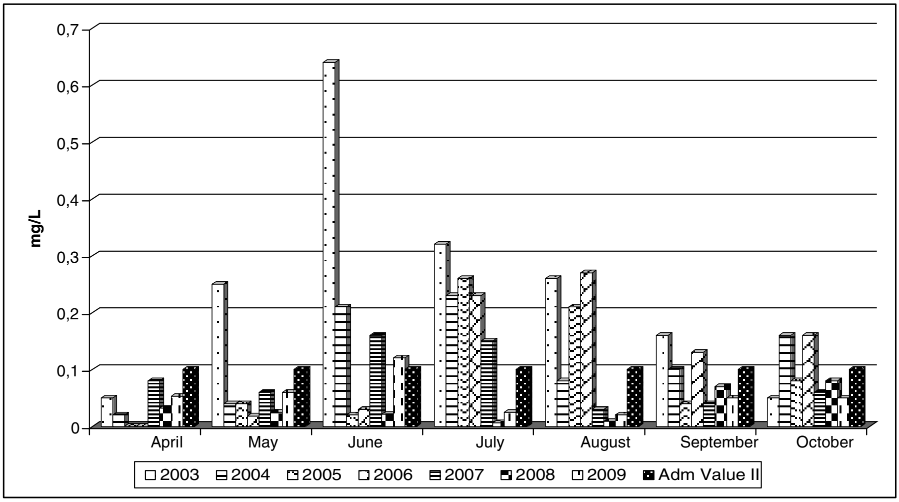

Figure 4. The Manganese concentrations in surface waters collected from Murighiol Location.

3.2. SEDIMENTS

3.2.1. *Total Content of Metals*

The values of total metallic concentrations were compared with the reference values for the sediment quality, according to the Romanian Norm 161/2006. In Tables 1 and 2 is presented a summary of the experimental data in order to obtain useful information. Thus, the tables contain the range of the values for the metals in all the investigated periods, the highest value recorded and the percentage of the samples that exceed the maximum admissible value. The metals Cd, Cu, Hg, Ni and Zn were found in concentrations higher than the maximum admissible values in both locations.

Based on the monitoring data obtained in the period 2003–2009, it has been observed that the concentration of Cu in sediment samples, collected from both locations, remained relatively stable, suggesting a historical anthropogenic contamination. In Uzlina Location, 85% of the samples exceeded the limit value while in Murighiol the percentage was 70.2%.

The same situation was concluded for high concentrations of Ni recorded in both locations (85.2% in Uzlina and 37% in Murighiol). High levels of Cu and Ni concentrations have been also reported in an assessment of element concentrations in Danube river sediments, carried out in 2001. Cu and Ni concentrations found along the river ranged from 31.3 to 662.9 mg/kg and from 24.6 to 142.8 mg/kg, respectively (Woitke et al., 2003).

TABLE 1. The total concentrations of metals in Murighiol Location in the period April to October of 2003–2009.

Metal	M.U.	Range of values	Number of samples	Percentage of exceeded values (%)	The highest value		Maximum admissible value
					Value	Month/ year	
As	mg/kg d.m.*	<0.1–12.3	43	0	12.3	06/2009	29
Cd	mg/kg d.m.*	<0.5–1.5	61	12.77	1.5	09/2005	0.8
Cr	mg/kg d.m.*	7.5–54.6	61	0	54.6	10/2005	100
Cu	mg/kg d.m.*	14.8–67	61	70.21	67	06/2005	40
Hg	mg/kg d.m.*	<0.1–0.79	43	14.29	0.79	09/2007	0.3
Ni	mg/kg d.m.*	19–111	35	37.04	111	06/2008	35
Pb	mg/kg d.m.*	7.5–51.3	61	0	51.3	09/2003	80
Zn	mg/kg d.m.*	29.8–216	61	10.64	216	10/2009	150

*dry matter

TABLE 2. The total concentrations of metals in Uzlina Location in the period April to October of 2003–2009.

Metal	M.U.	Range of values	Number of samples	Percentage of exceeded values (%)	The highest value		Maximum admissible value
					Value	Month/ year	
As	mg/kg d.m.*	<0.1–10.5	43	0	10.5	06/2009	29
Cd	mg/kg d.m.*	<0.5–1.5	61	10.64	1.5	07/2005	0.8
Cr	mg/kg d.m.*	19.7–61.9	61	0	61.9	07/2007	100
Cu	mg/kg d.m.*	23.2–194	61	85.12	194	07/2007	40
Hg	mg/kg d.m.*	<0.1–1.14	43	21.21	1.14	05/2008	0.3
Ni	mg/kg d.m.*	30.6–78.5	35	85.19	78.5	05/2007	35
Pb	mg/kg d.m.*	14.9–40.2	61	0	40.2	06/2003	80
Zn	mg/kg d.m.*	86.8–218	61	29.79	218	07/2007	150

*dry matter

Zn concentrations higher than admissible value were detected occasionally in Murighiol Location (10.6%) in July 2003, July 2006, September 2007, September, October 2009; the values were ranged from 153 to 216 mg/kg, in comparison with the admissible limit, which is 150 mg/kg.

In Uzlina Location, 27% from the sediment samples exceeded the limit value for Zn (July 2003, in all months of 2005, in May–August of 2006, in July 2007 and September, October 2009). The values were included between 158 mg/kg and 218 mg/kg.

Regarding to cadmium values, there are four sediment samples collected from Uzlina with high concentrations reported in June 2003, May 2005, July and August 2005. In Murighiol Location, six sediment samples exceed the limit, with values situated between 0.95 and 1.5 mg/kg.

The adsorbed Cr concentrations in sediment particles smaller than 63 μm, were in a relative uniform distribution within the scheduled investigation, in both locations and below the established criteria for the pollution.

Lead concentrations, in both locations were situated under the limit value in all months of the study.

Arsenic was below the determination limit of the analytical method used in almost the entire investigated periods. In 2009 were recorded As concentrations in both locations with values situated in the range 6.2 ± 12.3 mg/kg, well under the maximum admissible value of 29 mg/kg.

Mercury was found in the structure of sediment in 2005; the values were situated in normal levels of concentration until 2007. In Uzlina Location, the maximum admissible value was exceeded in the period July–October 2007 and April–June 2008 (0.47–1.14 mg/kg). In Murighiol Location, the problems regarding to Hg contents were recorded in the period of July–October of the year 2007 and also in June 2009 (0.36–0.79 mg/kg). The admissible value is 0.3 mg/kg for total Hg concentration in sediment structure according with Romanian Norm 161/2006.

The presence of As and Hg in the sediment structure starting from 2005 may be a cause of the strong floods, who affected the whole territory of Romania in 2005 and in the next years. This altered the hydrodynamic conditions of many rivers. The temporary high volume of water carried big quantity of alluviums and ensured the mixture of sediments, which explain the higher presence of As and Hg in the sediments structure in the period 2005–2009.

3.2.2. *Mobile Metallic Fraction*

In 2008, a comparative study of *mobile metallic fractions in wet and dried sediments* was performed in order to demonstrate the importance of the sediment pre-treatment procedure, when the samples are deposited in anoxic condition in natural environment. The sample preparation may generate undesirable changes of sediment properties (changes in metal availability and complexing properties) (Vasile and Tanase, 2010). The sediment samples were collected in the period from April to October of 2008 from Uzlina and Murighiol Locations and were analysed in wet/air-dried samples The exchangeable or mobile fraction corresponds to the form of metal that is most available for plant uptake and can be released by changing the ionic strength of the medium (Filgueiras et al., 2002).

In Figures 5 and 6 are presented the percentages of mobile fraction (average value for each element and location in all the investigated months of 2008) in wet sediment compared with the one obtained from air-dried sediment.

In terms of concentrations, in Table 3 are presented the range of values for each mobile element in sediments collected from Murighiol Location. The air-drying procedure applied to sediments collected from this site conduct to higher values of mobile copper with approximately 36% than the same fraction in wet sediments. The sulphuric fraction of metals, usually insoluble in anoxic condition, can be mobilized with the increasing of the oxidizing conditions. The process was reverse for Fe and Mn mobility. Mobile Fe content was eight times higher in wet sediment and mobile Mn was 2.5 times higher than in air-dried sediment. This different behavior can be due to an oxidizing process. In wet sediments, Fe and Mn are in higher concentration in ionic state, but in air-dried samples, part of mobile Fe and Mn was oxidized and bounded to organic matter and to the Fe-Mn oxide fractions.

The percentage values for Ni, Pb and Zn were in the same range in wet and in air-dried sediments and this fact proved that these elements were bounded in the sediment structure by the fractions not affected by air-drying procedure.

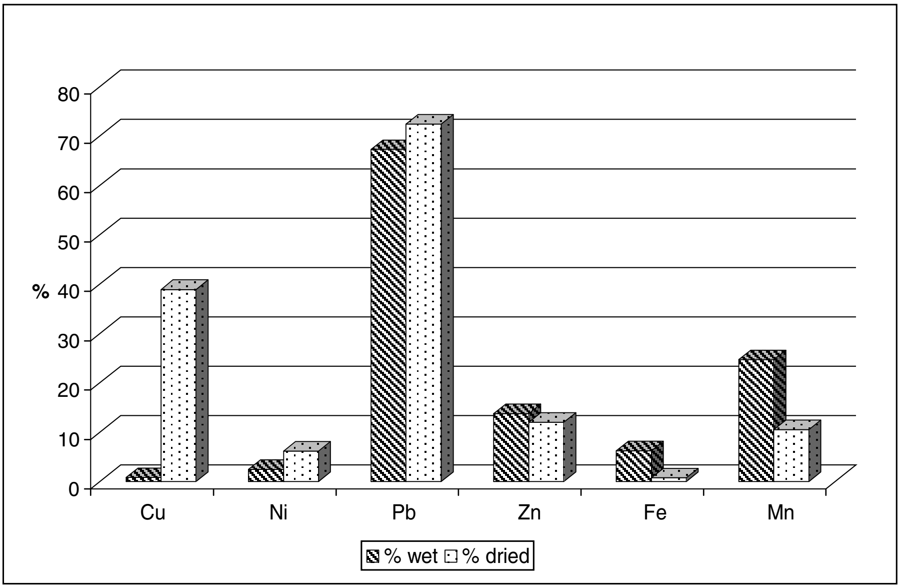

Figure 5. Comparative study of mobile metallic fraction in the wet/dried sediments collected from Murighiol location.

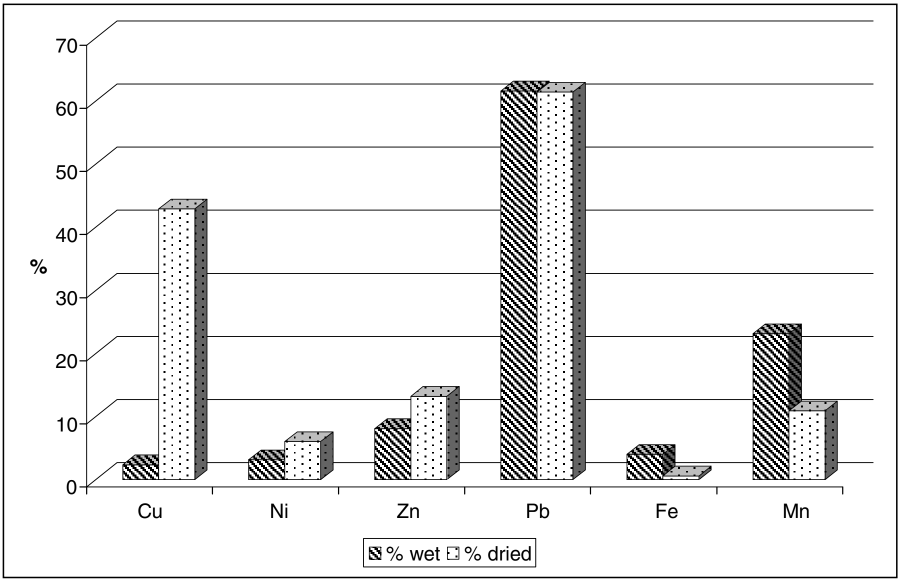

Figure 6. Comparative study of mobile metallic fraction in the wet/dried sediments collected from Uzlina Location.

TABLE 3. The concentration range for total and mobile metallic elements in Murighiol sediments in 2008.

Element	MU	Sediment			Maximum admissible value Norm 161/05
		Mobile fraction		Total content	
		Wet	Dried		
Cu	mg/kg d.m.*	0.1–1	15–17.3	41–46.4	40
Fe	mg/kg d.m.*	1,000–1,700	160–185	21,300–25,300	–
Mg	mg/kg d.m.*	95–145	45–58	452–586	–
Ni	mg/kg d.m.*	0.5–1	1.5–2.1	27.4–31.7	35
Pb	mg/kg d.m.*	15–21.5	20.1–22.1	21–37.5	85
Zn	mg/kg d.m.*	6–21	11.7–11.3	96–122	150

*dry matter

The values for mobile Cu and Ni in wet sediment indicate that Cu and Ni are not in ready-available forms for the surrounding water. If air-drying procedure is used for these types of sediments, the conclusions can be different, because the mobile Cu represents approximately 40% from the total content (15–17 mg/kg dry matter). In addition, the mobile content of Fe and Mn can be reported at lower values than they really are in wet sediment.

If the values of the total metallic content were compared with the reference values for the sediment quality, according to the Romanian Norm 161/2006, the total Cu concentration is higher than the maximum admissible value. In accordance with the presented mobility data, Cu was bounded to the fraction with low mobility and does not represent a possible pollution risk for the water column.

In Uzlina Location, a similar situation to the Murighiol sediments was recorded. The sediment structure presented a low mobility of Cu and Ni in wet sediment (natural condition of the studied environment). In air-dried sediments, the percentage of mobile Cu was 18 times higher than in wet sediment, 6.8 times lower for mobile Fe and two times lower for Mn fraction. These drastic changes reported in air-dried sediments are due to the oxidizing effect of the atmosphere conditions.

The percentages of mobile fraction in wet sediment, compared with air-dried sediment, are presented in Figure 6.

Conclusions could be withdrawn only when using the information from Table 4, with regard to the concentration range of total and mobile metallic elements from Uzlina sediments. The sediment structure from Uzlina Location, in wet conditions, presented low mobility of Cu and Ni and high concentrations of mobile Fe and Mn. The drying process did not affect the fraction of mobile Pb. A small increase of mobile Zn concentrations was reported in air-dried sediment (1.6 fold higher).

As a conclusion of experimental data obtained in this study, the mobile concentrations of Cu and Ni were reported to be low, as these elements have been bound in the fractions with high stability.

The large amounts of Pb found in mobile fractions in sediment samples collected from both locations, being more than 60% from the total concentrations, may have significant adverse impact on chemical and ecological status of the river water, especially during the low-flow seasons.

A Hg mobility test was started in 2008 in order to establish if the mercury found in the sediment structure can affect the biota and water body. Two different methods were used to check if any content of organic or inorganic Hg can be leached from the sediments. The study performed on wet polluted sediments collected from both Locations indicated that neither anorganic Hg (as $HgCl_2$, $Hg(OH)_2$, $Hg(NO_3)_2$, $HgSO_4$, HgO), nor organic Hg (CH_3HgCl, CH_3CH_2HgCl) were founded.

TABLE 4. The concentration range for total and mobile metallic elements in Uzlina sediments in 2008.

Element	MU	Sediment			Maximum admissible value Norm 161/05
		Mobile fraction		Total content	
		Wet	Dried		
Cu	mg/kg d.m.*	0.25–3	22–27	42–74.5	40
Fe	mg/kg d.m.*	560–1,300	125–175	24,100–25,300	–
Mg	mg/kg d.m.*	135–160	47–90	602–729	–
Ni	mg/kg d.m.*	0.75–1.50	1.6–3.8	30–69	35
Pb	mg/kg d.m.*	11–15.5	12.9–14.7	16.5–31	85
Zn	mg/kg d.m.*	7–12	15.1–18.8	111–145	150

*dry matter

3.2.3. *Sediment Metallic Structure*

The proportions of metals bound by different fractions in the sediment structure were established in 2004 in a single chemical extractions study. The sediments for the study were collected from both Locations in the period April–September of 2003 (Vasile et al., 2005).

The chemical partitioning of metals traces in the sediment samples among the exchangeable, Fe/Mn oxides, organic matter and residual fractions, is plotted in Figures 7 and 8 as a mean value of the metal concentrations in samples collected in each sampling point in 2003). Each geochemical fraction is presented as the percentage of the sum of all fractions. Irrespective of survey and with few exceptions, the chemical speciation of traces showed rather similar distribution patterns within sampling sites.

Copper appears to be associated mainly with Fe/Mn oxides, residual phase and organic matter.

Zinc remained mainly associated with residual phase, but is found in all other fractions. Cr is bound by poorly crystalline iron – oxides and organic matter fraction in different proportions. The most part of Cr is bound in well crystalline iron – oxides and residual fraction. The mobility tests indicated that Cr is not bound by the exchangeable fraction and is not available for the surrounding environment (biota and water body).

Lead preferentially was bound in Uzlina and Murighiol sediments in two fractions: mobile fraction, manganese oxides and organic matter fraction. High percentage (41% in Murighiol and 25% in Uzlina samples) of mobile Pb can be a source of possible threat to the water quality if occur some changes in the system stability (discharge of reductants or oxidants in the surface water, mechanical mixing of the sediment and water in natural flood or anthropogenic disaster).

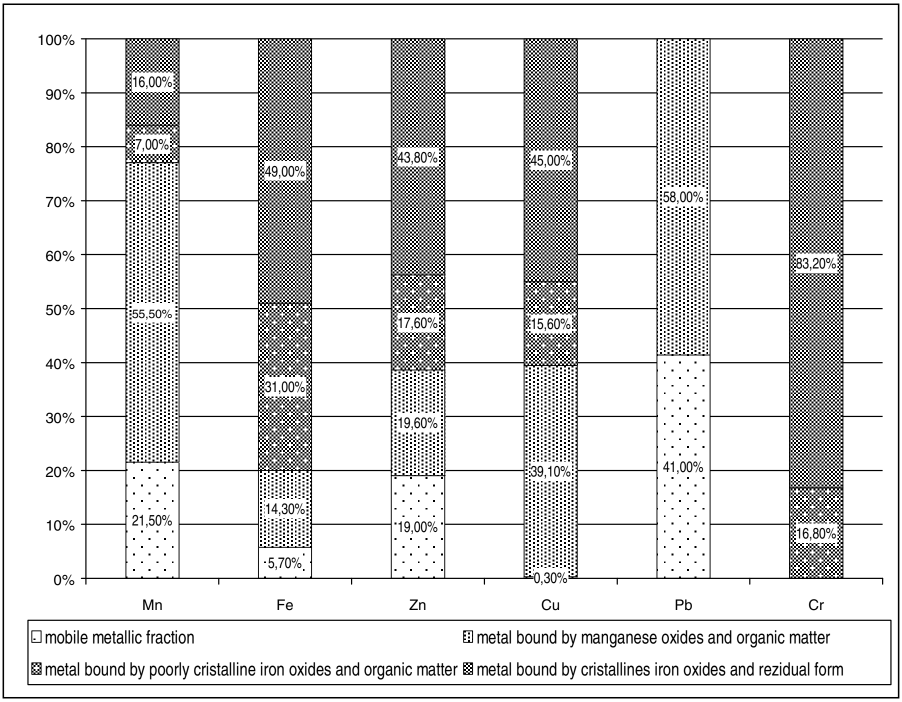

Figure 7. The sediment metallic structure in sediments collected from Murighiol Location.

Four metals were found in mobile form: Fe (4.5% from the total concentration in Uzlina, 5.7% in Murighiol), Mg (27% in Uzlina, 22% in Murighiol), Zn (11% in Uzlina, 19% in Murighiol) and Pb. Small quantity of mobile Cu, less than 1%, was found in both locations. Overall, the relatively high proportion of traces associated with the extractable fractions is indicative of significant heavy metal input of anthropogenic origin and of high potential mobility. These results are in accordance with the conclusions of similar studies carried out on sites affected by heavy metal pollution in different European rivers, e.g, the Latvian rivers (Klavins et al., 2000), the Louro River (Filgueiras et al., 2004) or Po River (Farkas et al., 2007).

In a study, concerning aquatic macrophytes, invertebrates and fish (Tudor et al., 2006), were found high levels of toxic metals such as Pb and Cd, which were acummulated in fish muscles. Higher Pb mobility inside sediment stucture may explain the presence of metal in fish species like: zander, perch and gibel crap. In macroinvertes and aquatic macrophytes the same study introduces a generalised concentration gradient of metal concentrations as follows: Cd < Pb < Cu < Zn.

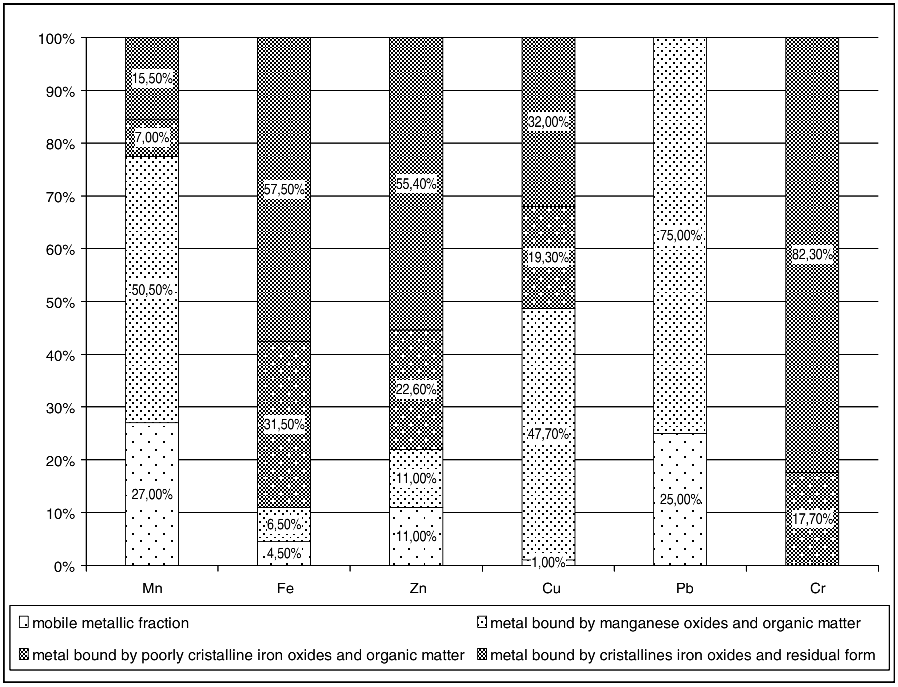

Figure 8. The sediment metallic structure in sediments collected from Uzlina Location.

AXIOS/VARDAR RIVER VALLEY

1. Introduction

Another important transboundary Balkan river with ecological and chemical status influenced by strong economic development is Axios/Vardar River (Figure 9).

Vardar River flows into the North Aegean Sea as Axios River in Greece (87 km long, extended over 3,212 km^2) and covers 23,747 km^2 (86.9%) of FYR Macedonia; small catchments parts are further in Serbia and Montenegro. The Vardar (Axios) river is the second in length river of the Balkan area and the longest and largest river of FYR Macedonia (302.6 km) whose floodplain is mainly used in agriculture. The capital of FYR Macedonia and several big industrial cities with a total population of over 1 million are located in this area: Gostivar, Tetovo, Scopje, Veles and Gevgelija, including several large units of chemical industry. The Vardar River Basin and its water resources are important in FYR Macedonia for irrigation, energy production, drinking water, industrial water and as a conduit for disposal of wastewater.

Figure 9. Map of Axios – Vardar River Valley.

Approximately 80% of the industry of FYR-Macedonia is located along the Vardar River and its basin banks as well as the mines of metallic and nonmetallic ores and coal, along with several hundreds of minor, middle and major production companies.

Steel plant in Skopje and lead smelter plant in Veles in the middle of the country, have been polluted the surface waters of the Vardar River more than three decades by heavy metals – iron, manganese, lead, cadmium and zinc (Vosniakos et al., 2007b).

Axios (Vardar) is also one of the most important transboundary rivers in Greece, mainly because of the use of waters for irrigation in the fertile plain of Thessaloniki and being a resource for industrial/municipal water. The activities on Greek side are mainly agricultural.

Smaller rivers (the Axios River tributaries) from the Prilep and Bitola areas diffuse pollutants to the Axios River with industrial and agricultural origin. In the Veles area, there is lead, zinc and fertilizer factories, which lead to pollution reached sometimes the Thermaikos gulf.

In Greek territory, the Axios River is also polluted by industrial, urban and agricultural waste, while its river delta presents some other significant problems related to its protection status (Albanis et al., 2009a; Albanis et al., 2009b)

In its estuaries to the Thermaikos gulf (located in the greater area of Thessaloniki – Greece), the Axios river forms an extended delta together with the Aliakmonas and Loudias rivers. This river delta is very rich in flora and fauna wetland and is protected by the Ramsar Convention.

The surface water quality has been classified as good/moderate at some points along the Axios River except Akropotamus, Polykastro and the outfall of the river where the water quality has been classified as poor.

In the frame of the ARCHIMEDES II Research Project regarding "Evaluation of the Transboundary Pollution of the Axios River – Direct and Indirect Impact to Man and Environment", in the period 2005–2006 were collected surface water and sediment from Axios (Vardar) River.

In the territory of FYR Macedonia, in order to assess the potential pollution from the Steel Factory and impact from the heavy traffic in Skopje, samples of surface water and sediment of the Axios (Vardar) River were taken at 5 km downstream near village Gorno Lisice.

Potential impact of heavy metals from Veles, and especially from the Lead smelter plant was assessed by examination of heavy metals in river water and sediment 20 km downstream of Veles, near Gradsko (Albanis et al., 2009a; Albanis et al., 2009b).

The sampling points in Greece were near to the Greek borders (East Prohoma, West Prohoma) and near to the river delta (East Malgara, West Malgara) (Vosniakos et al., 2007a; Vosniakos et al., 2007b).

At the same time were analyzed metals from some agricultural products (spinach, young onion, green salad, tomato, eggplant, paprika, bean-pounds, peas, celery, lettuce, carrots and parsley) in order to observe the potential pollution of these in river floodplain.

2. Experimental Data

2.1. METALS

In the river water on the territory of FYR Macedonia, there were no differences between the two measuring sites (village Gorno Lisice and Gradsko) related to the levels of Pb, Cd, Cu, Zn, As, Cr and Ni. However, concerning the concentration of Mg downstream of Veles, almost twice higher concentrations have been found when compared to village Gorno Lisice.

By examining metals in the river sediment, it has been found that Cr has higher concentration at the measuring site village Gorno Lisice. That could be explained by pollution of the river by Cr a few decades ago upstream of Skopje from Factory Jugohrom Jegunovce. Despite the fact that Cd levels were low, data have shown almost fourfold higher concentrations downstream of Veles compared to village Gorno Lisice, probably because of the impact from the Lead smelter plant in Veles. Zn was almost twice higher downstream of Veles, compared to village Gorno Lisice. In April 2005 Pb has been tenfold higher downstream of Veles, compared to village Gorno Lisice. The concentrations of Hg in the sediment structure collected from Gorno Lisisce site ranged from 0.24 to 0.55 mg/kg, while in Gradsko the contents of Hg were higher (from 0.21 to 0.84 mg/kg) (Figure 10).

In 2005, at the measuring site near village Gorno Lisice, almost all samples of agricultural products taken in each season have been polluted with Hg; the values were situated between 0.01 and 0.13 mg/Kg (Figure 11). Eight samples in each season were polluted by Cd (spinach, young onion, celery and lettuce), and only one by lead (eggplant).

Concentrations of lead and cadmium were relatively higher at the measuring site in Gradsko (Figure 12). The content of mercury was ranged from 0.01 to 1.25 mg/kg, bigger than maximum admissible value, which is 0.02 mg/kg, in almost all agricultural products. The highest concentrations of Hg were recorded in carrots (1.25 mg/kg) and in beans (0.46 mg/kg).

In 2006 were found high concentrations of Hg in both locations in lettuce and parsley products (Figure 13).

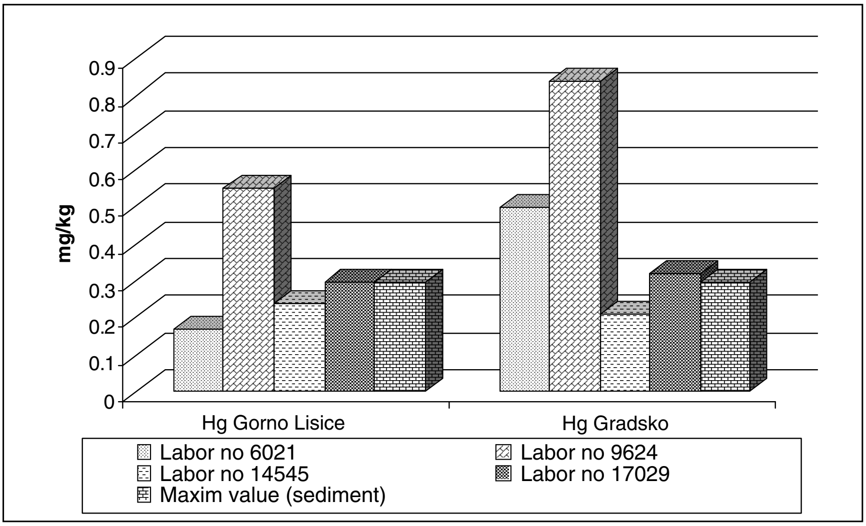

Figure 10. Mercury concentrations in sediment samples collected from Vardar River.

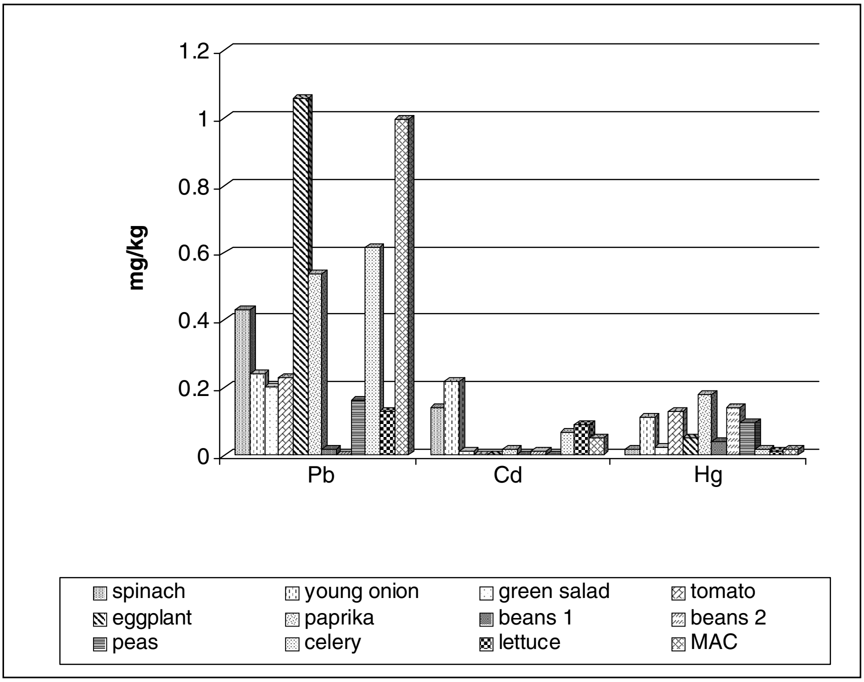

Figure 11. Heavy metal concentrations in agricultural products near Vardar – Gorno Lisice (5 km downstream of Skopje) in 2005.

The levels of Pb, Cd and Hg in the agricultural products downstream from the Lead smelter plant could influence the potential impacts on the environment and indirectly on the human health through the food chain. It can be assumed that there is an environmental risk, possibly because of irrigation with water from the river, and most likely because of the impact of pollution with particulate matters through the ambient air.

In the Greek part of the Axios River were collected surface water samples. The content of heavy metals was determinate and the data shows high concentrations of iron, zinc and aluminum waste from the areas of North Greece and FYR of Macedonia (Albanis et al., 2009b).

According to the data obtained in the project (Vosniakos et al., 2007a; Vosniakos et al., 2007b), the River Axios on the territory of the Veles municipality has a high self-cleaning potential, which however is not enough to abolish the presence of the very high biochemical pollution by the local industry. It can be assessed that in long-term process wastewaters will decrease the quality of the surface waters as in the region of Veles, as well downstream in the other parts of the FY Republic of Macedonia.

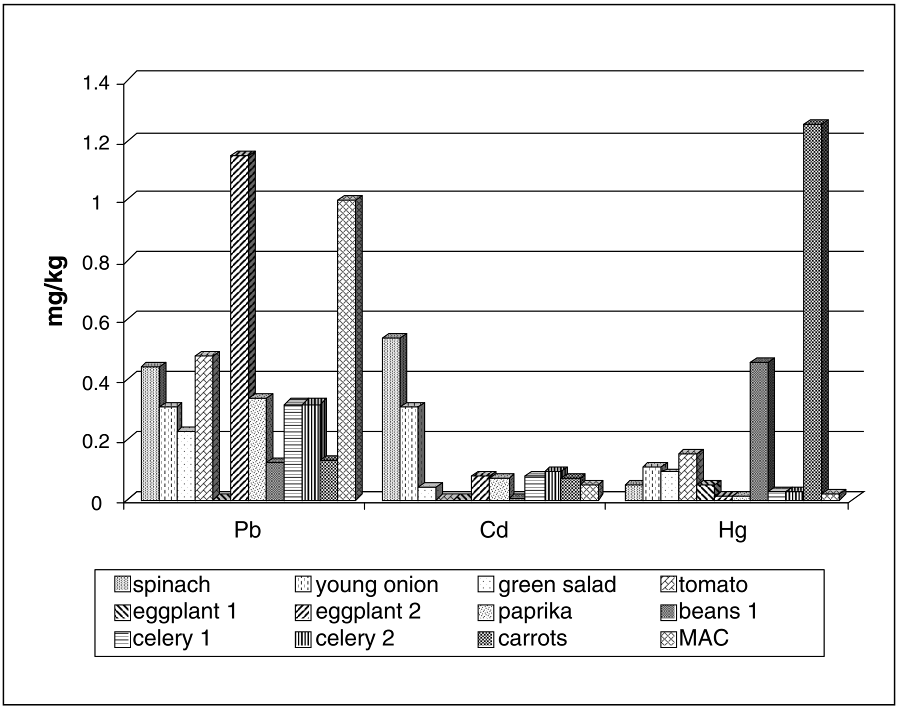

Figure 12. Heavy metal concentrations in agricultural products near Vardar – Gradsko (20 km downstream of Veles) in 2005.

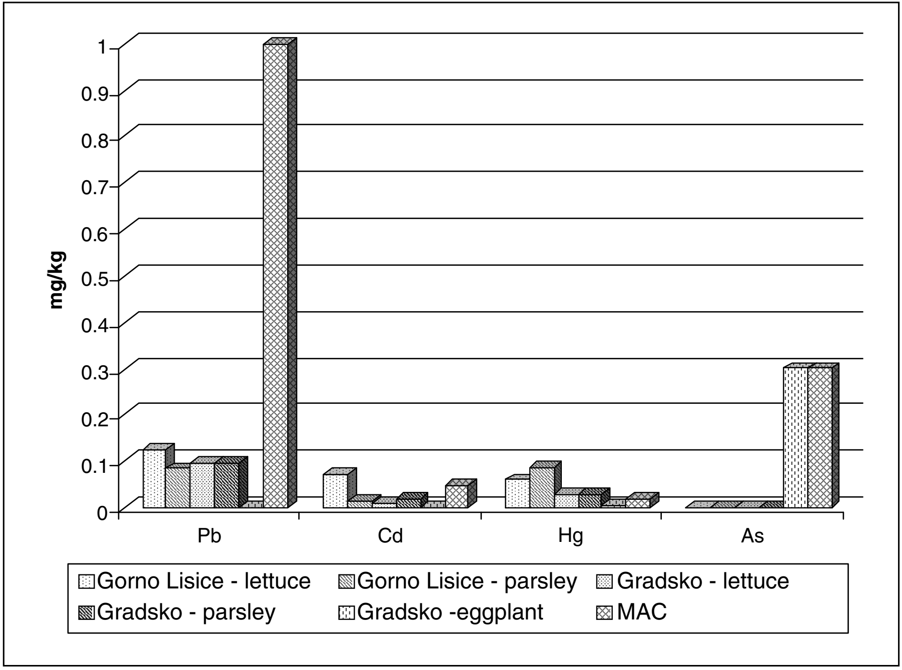

Figure 13. Heavy metals concentration in agricultural product near Vardar River in 2006.

The results shown a continuous degradation of the quality of the surface water of the Axios/Vardar basin, which is mainly due to the toxic organic substances (pesticides, PCB) and to the high concentration of some metals and other inorganic pollutants such as phosphates and nitrates. The agricultural pollution is also significant in both countries, Greece and FYR of Macedonia.

3. CONCLUSIONS

The pollution of surface water and sediments on St. Gheorghe branch from the Danube Delta with nutrients, metals and several relevant organic toxic compounds was evaluated. The variation of parameters during the investigated period in Uzlina and Murighiol was strongly influenced by the climatic conditions (drought, flood). In springs of 2003 and 2004, when it was a very dry weather without rains, highest values were recorded in Murighiol for: organic load (BOD, 118 mg O2/L; COD, 295 mg O2/L), June 2004; iron (8 mg/L) in June 2003; manganese (0.64 mg/L) in June 2003.

In spring–summer time of 2005 and 2006, a better quality of the surface water was recorded, especially because of strong dilution of water. However, in autumn, it was noticed a return to the normal flow of water, which modified the quality of surface water in a negative way.

In surface water, the metal load of the water-body has been generally low; exceptions were Fe and Mn concentrations, which exceeded the limit values almost every month.

From all metals studied, Cd, Cu, Hg, Ni and Zn represent a particular concern because of their high total concentration levels found in the sediments. The large amounts of Fe, Mn and Pb found in mobile fractions of the sediment samples may have significant adverse impact on chemical and ecological status of the river water, especially during the low-flow seasons.

In conclusion for both rivers Danube and Vardar/Axios, the measured values were momentary values and show the character of the contamination levels, singular, relating to the period and locations of the investigation campaign development.

The pollutants, found out in concentrations that exceeded the limits, represent a potential for water quality alteration. The negative influences on auto purification of the rivers imply the diminution of the biological activity. Generally, for all investigated periods we point out that in both locations, the watercourses presented a deficit in oxygen, and under saturation, do not satisfy the prescribed regulations (6 mg/L) concerning the conditions for aquatic organisms' protection.

The results of analytical studies performed to observe and control the surface water quality of the two major rivers that cross the South-East Europe showed the existence of a dynamic pollution with toxic metals and organic compounds.

The presence of Fe, Mn, heptachlor, Lindane, PCBs, phenols, mineral oil in surface waters represent an acute problem for the inhabitants of the Danube Delta and Vardar/Axios basin. The use of water for household consumption directly from these rivers, without any treatment, presents a series of disadvantages, such as a high risk of diseases for the population, mostly for children and old people.

Target analyses of selected pollutants showed that occasionally they were present in high concentration in the investigated samples, and revealed the need to safeguard the quality of the river water from future deterioration, firstly by a long-term monitoring. Further studies need to be carried out also on the biota compartments like fish from this area in order to evaluate the occurrence of persistent organic pollutants and toxic metals in tissues, and to assess the risk of these contaminants on the ecosystem and human health.

These conclusions show a dynamic variation of physical – chemical characteristics of watercourses in the investigated locations and the necessity to continue the study in the coming years.

The final conclusion is that the behavior of the two rivers is similar in the base of comparison, regarding their pollution and their seasonal physico-chemical characteristics.

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ATMOSPHERIC HEAVY METALS POLLUTION: EXPOSURE AND PREVENTION POLICIES IN MEDITERRANEAN BASIN

MAHMOUD A. HASSANIEN*
Air pollution Department, National Research Center
El Boohos St., Dokki, Cairo, Egypt

Abstract. Atmospheric heavy metals pollution is one of the most serious problems facing humanity and other life forms on our planet today. Industrial pollution, soil erosion, deforestation, rapid industrialization, urbanization, and land degradation are all worsening problems. The release of toxins from plants and industrial institutions is very high for the per capita allowance. In some Mediterranean countries, the combustion of wood or agricultural waste is another major source. Present information concerning the sources, emission, limits and inputs of heavy metals is rather limited in the developing countries. The situation in developing countries (North African and Middle East) is mixed. In these countries, however, and in some economies in transition (including Eastern Europe) traffic is becoming the problem. This is a challenge to city planning in these countries, where the long repressed wishes for private automobiles are difficult to reconcile with environmental protection. These countries are seeking to expand their economic activities; consideration for environmental conservation often receives a low priority. In addition, approaches used in industrialized countries often cannot be applied directly in developing ones. For any industry, the fate of the air quality is far down the list beyond their ability to make a profit. Exposure assessment studies in the developing world are important. Differences among measuring methods and a lack of strict quality control in carrying out exposure assessment make it difficult to generalize and compare findings between studies. Toxic chemicals can be transported with differing levels of efficiency to the target host depending upon the transport pathways. Exposure may occur directly by ingestion, inhalation, or dermal contact. The relative contribution of different pathways must be assessed by examining the nature of human activities which may be expected in particular exposure settings. This chapter summarizes some of the basic principles and uses of environmental risk assessment. Exposure assessment requires the integration of environmental quality data with an estimate of the rate of human contact with

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* To whom correspondence should be addressed: mhassanien@gmail.com

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contaminated media. This aspect of risk assessment should rely heavily on local data, since it allows an assessment of how particular local conditions and cultural practices affect risk potential. A number of site-specific factors must first be evaluated, including (1) the characteristics and quantification of industrial pollutants, (2) the potential for release to the environment, (3) the sensitivity of the particular environment, (4) the proximity of these chemicals to humans, and (5) its potential effect on human health.

Keywords: Air pollution, particulate matter, heavy metals, Mediterranean countries, exposure, preventative strategies

1. Background

Air pollution is caused mainly by the combustion of petroleum products or coal by motor vehicles, industry, and power stations. In some Mediterranean countries, the combustion of wood or agricultural waste is another major source. Pollution can also originate from industrial processes that involve dust formation e.g., cement factories and metal smelters (Hassanien, 2009). The Mediterranean basin has never been a major mining region and thus was not involved in the period of industrial development based on coal and iron. It is better endowed in oil and natural gas (Algeria, Egypt, Libya, Syria and Italy), leading to establishment of many refineries all around the Mediterranean basin (Gullu et al., 2005). Indoor sources also contribute to outdoor air pollution, and in heavily populated areas, the contribution from indoor sources can create extremely high levels of outdoor air pollution. Emissions from these sources pollute the air we breathe. Recent results suggest that adverse health effects of air pollution exist at levels of pollutants around or even below air quality standards set by national and international institutions.

Furthermore, there are indications that air pollution effects on health may be partly determined by specific mixtures of air pollutants and may be altered by other environmental, behavioral, and social patterns. Southern European countries share some common characteristics in terms of climate, geography, and life activity patterns. Results from studies undertaken in France, Greece, Italy, Portugal, and Spain investigating short- and long-term air pollution health effects are presented and their consistency demonstrated. These results provide adequate evidence that health effects-particularly short-term-of the currently measured urban air pollution levels exist. However, information available so far does not allow an assessment of regional differences in the health effects of air pollution as far as the Mediterranean region of Europe is concerned. Evidence shows that air pollution at current levels in Mediterranean cities is responsible for a significant burden of disease, mortality, hospital admissions and exacerbation of symptoms, especially in relation to mental and cardiovascular diseases. Very young children and pregnant woman are especially vulnerable to air pollutants (WHO, 2007).

Outdoor and indoor air quality is important to human health. The pollutants can be divided into two groups, the traditional major air pollutants (MAP, comprising sulfur dioxide, particles, nitrogen dioxide, carbon monoxide, lead, and ozone) and the hazardous air pollutants (HAP, comprising chemical, physical, and biological agents of different types) (Wiederkehr and Yoon, 1998). The HAPs are generally present in the atmosphere in much smaller concentrations than the MAPs, and they often appear more localized (typically in urban areas or near industries), but they are—due to their high specific activities—nevertheless toxic or hazardous. Both in basic investigations and in abatement strategies HAPs are difficult to manage, not only because of their low concentrations but also because they are in many cases not identified (Morawska and Salthmmer, 2003).

Heavy metals such as iron (Fe), vanadium (V), chromium (Cr), cobalt (Co), nickel (Ni), manganese (Mn), copper (Cu), selenium (Se), barium (Ba), gallium (Ga), caesium (Cs), europium (Eu), tungsten (W) and gold (Au) exist in both coarse and fine fractions in ambient air (VIC EPA, 1998a). Calcium (Ca), aluminium (Al), titanium (Ti), magnesium (Mg), scandium (Sc), lanthanum (La), hafnium (Hf) and thorium (Th) exist predominantly in the coarse fraction (Klee, 1984). Metals such as arsenic (As), cadmium (Cd), gallium (Ga), molybdenum (Mo), lead (Pb), antimony (Sb), selenium (Se), tungsten (W) and zinc (Zn) enrich the fine fraction of particulate matter (Finlayson-Pitts and Pitts, 1986). Heavy metals are natural constituents of the Earth's crust. They are stable and cannot be degraded or destroyed, and therefore they tend to accumulate in soils and sediments. However, human activities have drastically altered the biochemical and geochemical cycles and balance of some heavy metals. Relatively volatile heavy metals and those that become attached to air-borne particles (particulates) can be widely dispersed throughout the atmosphere, often being deposited thousands of miles from the site of initial release (Sternbeck et al., 2002; Birmili et al., 2006). In general, the smaller and lighter a particle is, the longer it will stay in the air. Larger particles (greater than 10 µm in diameter) tend to settle to the ground by gravity in a matter of hours whereas the smallest particles (less than 1 µm in diameter) can stay in the atmosphere for weeks and are mostly removed by precipitation.

2. History and Adverse Health Effects of HMs

Some metals are naturally found in the body and are essential to health. Iron, for example, prevents anemia and zinc is a cofactor in over 100 enzymes reactions in the body. In trace amounts these metals are beneficial to the body; however, in high doses they can be toxic. When trace metals have a density of at least five times that of water they are called "heavy" and are difficult if not impossible for the body to metabolize without concerted nutritional effort. The metals accumulate in the cells and tissues, interrupt cellular function, damage DNA and slowly destroy people's health if they are not removed from the body. The most common heavy or toxic metals are: mercury, nickel, lead, arsenic, cadmium, aluminum, platinum and copper (the metal form, not the ionic form required by the body). These metals have no function in the body and are hazardous to your health (Hewitt and Jackson, 2003).

Numerous studies confirm that toxic metals have damaging effects on all the cells of the body and directly impair neurological, immune, endocrine, digestive, and respiratory and detoxification functions of the body. Most of this damage is a result of the oxidative damage done to cells from the toxic metals (Lippmann, 2009). Even minute amounts of heavy metal exposure can negatively impact your health, but the following factors determine to what degree you will suffer:

1. Nutritional status, especially anti-oxidant levels such as vitamins C, E and beta carotene, selenium and glutathione levels.
2. Strength of an individual's detoxification organs: colon, liver, kidneys and lungs.
3. Quantity and duration of the toxic exposure.
4. Immune system strength.
5. Other toxic exposures such as chemicals and radiation levels.

3. Sources of Atmospheric Heavy Metals

Heavy metals (HMs) released into the atmosphere from the anthropogenic sources represent part of the pollutant agents which may be responsible of serious problem to the public health. Depending of their concentrations and their chemical and physic-chemical forms adverse and/or toxic effects are well established for many trace metals that can be released in the air from different pollution processes (Rizzio et al., 2001 and Crutis et al., 2006). Metals are more frequently associated with air pollution particles of anthropogenic origin (Song et al., 2001; Wang et al., 2005; Al-Momani et al., 2005). Such particles originate from the incomplete combustion of carbon-containing materials at power plants, smelters, incinerators, cement kilns, home furnaces, fireplaces, and motor vehicles. Moreover, the major sources of heavy metal pollution in urban areas of North African countries are anthropogenic, while contaminations from natural sources predominate in the rural areas. Anthropogenic sources of pollution include those associated with fossil fuel and coal combustion, industrial effluents, solid waste disposal, fertilizers and mining and metal processing (D'Almedia et al., 1991; Pacyna, 1984; Sweet and Vermette, 1993; Sullivan and Woods, 2000). At present, the impact of these pollutants is confined mostly to the urban centers with large populations, high traffic density and consumer-oriented industries. Natural sources of pollution include weathering of mineral deposits, brush burning and windblown dusts.

The most significant sources of HMs include:

- Metals industry (Al, As, Cr, Cu, Fe, Zn);
- Other manufacturing industries and construction (As, Cd, Cr, Hg, Ni, Pb);
- Electricity and heat production (Cd, Hg, Ni);
- Road transportation (Cu and Sb from brake wear, Pb and V from petrol, Zn from tires);
- Petroleum refining (Ni, V);
- Phosphate fertilisers in agricultural areas (Cd).

4. Measurement Specific Metals in Air Pollution Particles

Metals occur in air in different phases, as solids, gases or adsorbed to particles having aerodynamic sizes ranging from below 0.01 µm to 100 µm and larger (Bouble, 1994). Toxicity of particulates depends greatly on their size, with particulates less than 10 µm (PM_{10}) or 2.5 µm ($PM_{2.5}$) being considered especially dangerous since they can easily penetrate the lungs into the alveoli. Currently there is some question as to whether the PM_{10}, $PM_{2.5}$ or $PM_{2.5–10}$ is more important to human health (Brunekreef and Forsberg, 2005). Table 1 ranks the emission of different metals in flay ash from the various industries. Two major categories of particulate matter are fine particles and coarse particles. Fine particulate matter (FPM) comprises particles with aerodynamic diameters of 2.5 µm or less. They are emitted from fossil fuel combustion, motor vehicle exhausts (including diesel) and wood burning. Several toxic metals, including arsenic, cadmium, lead, zinc, antimony and their compounds are associated with FPM in ambient. This is important from a public health perspective since these fine particles are respirable and can be transported over very long distances (Al-Masri et al., 2006; Santoso et al., 2008).

To measure metals, air pollution particles must be collected. This is typically accomplished by employing either a filter or inertial separation. A number of specific, sensitive and accurate standardized techniques are available to quantify metal concentrations. Figure 1 shows the steps for analysis of airborne particles.

However, there is no convention for choosing the procedures used to extract the metals for analysis. Atomic absorption spectrometry is the most widely employed technique for elemental analysis of airborne metals. Inductively coupled plasma emission spectroscopy also can be used for the simultaneous determination of up to 20 metals. Neutron activation analysis also can provide a nondestructive determination of up to 35 elements with high precision and accuracy. Finally, X-ray fluorescence spectrometry is a non-destructive technique that can simultaneously determine up to 30 elements. A major limitation of these analytical techniques is that they provide information on total metal content rather than specific compounds or chemical species.

TABLE 1. Metals present in fly ash of different industrial origin.

Source	Metals present in fly ash
Smelters	Fe, Cd, Zn, Pb
Oil-fired power plants	V, Ni, Fe
Coal fired power plants	Fe, Zn, Pb, ZV, Mn, Cr, Cu, Ni, As, Co, Cd, Sb, Hg
Municipal waste incineration	Zn, Fe, Hg, Pb, Sn, As, Cd, Cd, Co, Cu, Mn, Ni, Sb
Open hearth steel furnaces	Fe, Zn, Cr, Cu, Mn, Ni, Pb

5. Heavy Metals Levels in Aerosols

One objective of this chapter is, using collected data across Mediterranean countries as an example, to provide a demonstration of how the heavy metal contents of urban aerosols vary greatly depending on the environmental setting, irrespective of the average PM mass measured at a given site. The observed chemical variations in trace metal compositions are controlled by the relative contributions from traffic versus industry, the type of industry, the prevalence of resuspension processes, and the frequency of exotic dust intrusions (Querol et al., 1998; Sternbeck, et al., 2002; Sun et al., 2004; Moreno et al., 2005, 2006a, 2006b).

The metals in air pollution particles originating from natural sources vary with the source of the particles (Schroeder et al., 1987). Those metals in highest concentrations in crustal dust (in order of abundance) are iron, manganese (550 μg/g), zinc, lead, vanadium, chromium, nickel, copper, cobalt, mercury and cadmium. While the ocean generally acts as a sink for toxic metals, ocean aerosol contains concentrations of metals which are very low, with the highest being iron (51 μg/g), manganese, lead, vanadium (0.0009 μg/g) and zinc. Volcanic ash can have great amounts of iron, manganese, vanadium, zinc, cobalt, arsenic and antimony (in decreasing order of importance).

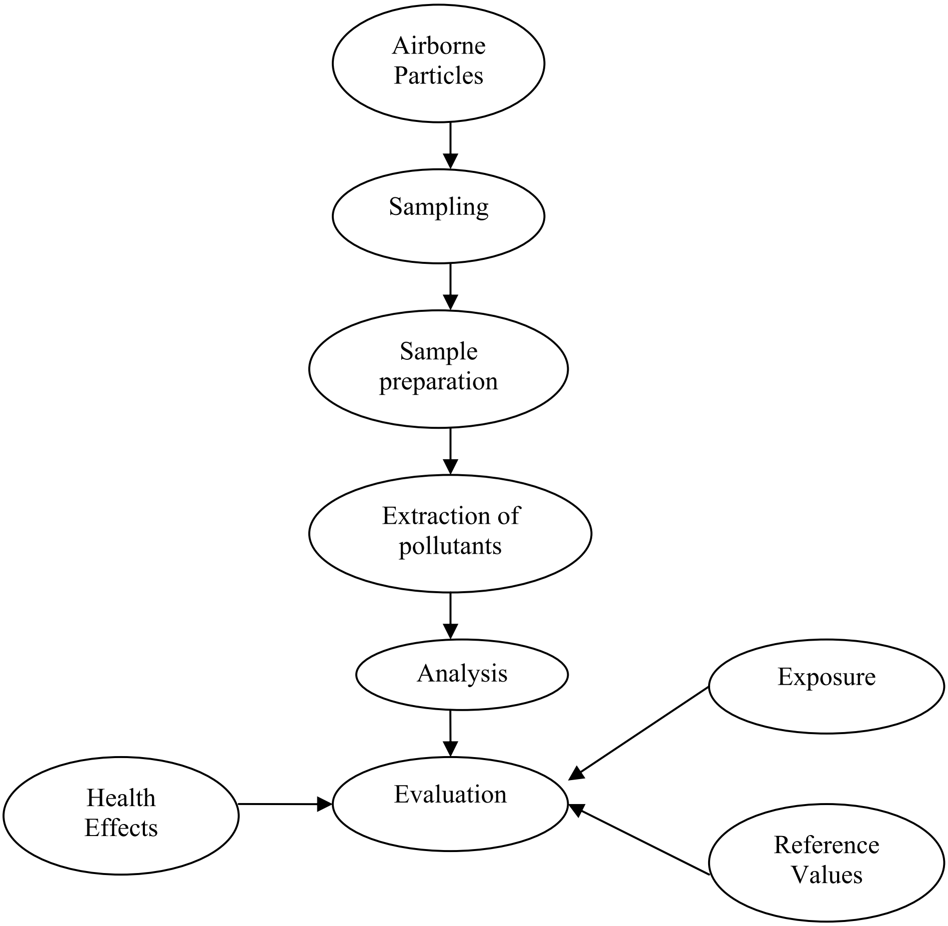


Figure 1. Steps for analysis of airborne particles using sample collection methods.

The lifetime of aerosols in the air, which contain heavy metals, is a function of the particle size. The smallest particles, 0.001–0.08 µm, have a lifetime of <1 h, because of coagulation into bigger particles, whereas in accumulation range, 0.08–0.1 µm, the lifetime is 4–40 days, and the large particles >1.0 µm have a lifetime of minutes to days (Caggiano et al., 2010). Since they are associated with particles, residence times of metals in the atmosphere are short, typically less than 40 days. Transport of metals nevertheless occurs over long distances, causing perturbations of ecosystems on a global scale (Nriagu and Pacyna, 1988).

Evidence shows that atmospheric heavy metals (HMs) at current levels in Mediterranean cities are responsible for a significant burden of disease especially in relation to mental diseases. Very young children and pregnant woman are especially vulnerable to air pollutants (WHO, 2007). At present, the impact of these pollutants is confined mostly to the urban centers with large populations, high traffic density and consumer-oriented industries.

Recent results suggest that adverse health effects of HMs exist at levels of pollutants around or even below air quality standards set by national and international institutions. Furthermore, there are indications that HMs effect on health may be partly determined by specific mixtures of air pollutants and may be altered by other environmental, behavioral, and social patterns. In addition, measurements of individual exposure to different pollutants, affected by the pollutant's levels in specific micro-environments and the individual's time-activity pattern, must be undertaken for a better understanding of the air pollution-health link.

Most countries have established such limit values for the major air pollutants and use, in addition, guideline values for a series of other compounds. Most important are the limits in the EU and the USA, which in many cases have served as models for other regions. Overview study examples were carried out in some Mediterranean courtiers are summarized in the following section e.g., speciation of three trace elements (Zn, Pb, Cu) in air particulates of two Syrian cities (Tartous and Darya) with different climate conditions and industrial emissions has been studied during 2000–2001. Approximately 60% of lead in air particulates of both cities was found to be associated with organic materials produced by incomplete burning of vehicles fuels and residential heating; the remaining 40% of lead was as lead oxides and mineral acids soluble compounds. Lead concentration was ranged from 100 to 84 ng/m^3 in both sites lower than Syrian standards for air quality in urban area (SAOSM, 2004) which is 0.5–1 μg/m^3 (Al-Masri et al., 2006).

In Italy, PM_{10} and $PM_{2.5}$ samples have been collected at a rural site of south-east from June to October, 2004 to investigate natural and anthropogenic contributions on particulate matter and heavy metal mass concentrations. Al, Cd, Cr, Cu, Fe, Mn, V, Ni, Pb, and Zn metal concentrations have been evaluated by an inductively coupled plasma atomic emission spectrometer. Accordingly to geochemical calculations Al, Fe, and Mn, have a significant crustal origin while, Cd, Cu, Pb, and Zn are of anthropogenic origin. Moreover, Fe was predominant in the coarse particle fraction, while Ni, Pb, V, and Zn were predominant in the fine particle fraction. It was also shown that Cd, Mn, Ni, Pb, and V concentrations never exceeded guide and/or limit values recommended by the

World Health Organization and the European Council Directives. The represented results in Table 2 show the atmospheric heavy metals contents in two different Italian cities.

TABLE 2. Average values of HM concentrations (ng/m^3) for five years periods at a city and background site[*].

Element	Odense (city street)		Tange (background)	
	1982–1986	1995–1999	1982–1986	1995–1999
V	18.7	5.0	7.0	2.8
Cr	2.6	4.4	2.0	0.5
Mn	20.7	19.8	10.3	5.4
Fe	760.0	640.0	268.0	140.0
Ni	7.6	2.7	3.6	1.2
Cu	20.8	20.4	2.8	1.8
Zn	99.7	47.4	32.7	16.0
As	–	1.2	2.0	0.9
Se	0.4	0.7	0.6	0.5
Mo	1.1	1.4	0.3	0.2
Cd	0.6	0.6	0.5	0.3
Sn	2.4	1.4	1.4	0.7
Sb	3.2	5.6	1.1	0.7
Pb	572.0	15.7	38.9	7.6

[*] Source: Kemp (2002)

Currently more information available as to the deleterious effects of lead on children's mental development; the lack of any clear threshold and the uncertainty associated with the multiple uptake pathways for lead within this vulnerable group, it is recommended that the limit value be decreased to 0.5 µg/m^3 and that the criteria measurement should be the annual arithmetic mean 24 h measurement. This would be in line with the current EU air quality (AQ) directives and others as demonstrated in Table 3.

In Cairo, Egypt; Lead is a particular local problem in Cairo due to the archaic technology used in the smelting process. However lead pollution has been considerably reduced and is less widespread as lead is no longer used as an additive in gasoline (EEPP, 2004). The results from the 1999–2002 indicate that in Shoubra el-Kheima, an area where a number of secondary smelters and small foundries are located, arsenic, chromium, cadmium, and manganese are found at sufficient concentrations to pose significant toxic health effects. In addition, relatively high ambient concentrations of arsenic were measured in Zamalek (residential area). Arsenic and cadmium are also known carcinogens and were found in sufficient concentration to warrant concern. Table 4 provides data from the SAS (Source Attribution Study) sampling sites, showing the distribution of various heavy metals pollution. It should be noted that there has been a reduction of concentration of these metals associated with the closure and relocation of the largest of the lead smelters during 1999 (EEPP, 2004). *Figure 2.* illustrates lead concentrations in residential and traffic areas during 2005–2007, showing clear decrease in lead concentrations in such areas.

TABLE 3. Summary of existing air quality standards and guidelines for lead.

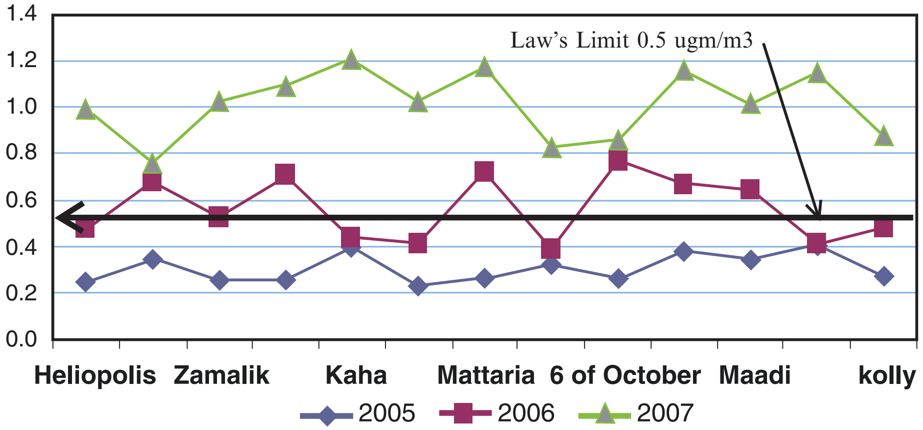
Agency	Guideline Value	Basis of Standard/Guideline
Ontario (MOE)	0.7 µg/m^3 (30-day) 2 µg/m^3 (24 h) 6 µg/m^3 (half-hour) AAQCs	Consideration of technical achievability and cost (MOEE, 1994b)
US EPA	0.15 µg/m^3 (3-month average NAAQS) (older limit was 1.5 µg/m^3)	Based on keeping 99.5% of children from exceeding a BLL of 40 µg/dL (US EPA, 2005; 2006a; Appendix J of Cal/EPA, 2001)
California/EPA	0.12 µg/m^3 (30-day average)	Based on an increase of 10% of the baseline average BLL in children in a defined highly exposed sub-population (Cal/EPA, 2001)
WHO/Europe	0.5 µg/m^3 (annual guideline)	98% of an exposed population should not exceed 10 µg/dL (WHO, 2000)
U.K.	0.5 µg/m^3 (annual mean objective for 2004) 0.25 µg/m^3 (annual mean objective for 2008)	0.5 µg/m^3 is based on WHO guideline. 0.25 µg/m^3 is based on including contribution of indirect[c] pathways (UK DETR, 1999)
New Zealand MFE	0.2 µg/m^3 (3-month average, measured in PM_{10})	Based on being consistent with UK's long term objective (NZ MFE, 2002)
Australia NEPC	0.5 µg/m^3 (annual average)	Based on the economic value of averted health impacts (Australia NEPC, 1998)

Source: Modified from Ontario Air Standards for Lead and Lead Compounds (2007)

TABLE 4. Metal concentration at different sampling sites in Cairo, Egypt*.

Site type	Mn (µg/m^3)	Cr VI (µg/m^3)	As (µg/m^3)	Cd (µg/m^3)
Rural	0.0911	0.0008	0.0023	0.0114
Industrial	0.2846	0.0022	0.0336	0.0155
Residential	0.1175	0.0011	0.0163	0.0173
Traffic	0.0975	0.0012	0.0076	0.0135
Mixed	0.1345	0.0017	0.053	0.0124

Source: (EEPP, 2004)



Source: (EEAA, 2008)

Figure 2. Lead concentrating in different areas in Cairo, Egypt.

In Spain, it was found that the highest average annual PM10 concentrations of all trace metals (total average trace metal: ΣTM_{10}) were registered at the Llodio (ΣTM_{10} 811 ng/m^3) and Barcelona (ΣTM_{10} 418 ng/m^3) sites, followed by Huelva (ΣTM_{10} 281 ng/m^3), Alcobendas (ΣTM_{10} 243 ng/m^3), Tarragona (ΣTM_{10} 175 ng/m^3) and Canaries (ΣTM_{10} 170 ng/m^3). In the fine fraction ($\Sigma TM_{2.5}$) the same pattern continued, with once again the highest concentrations being recorded at Llodio ($\Sigma TM_{2.5}$ 503 ng/m^3) and Barcelona ($\Sigma TM_{2.5}$ 210 ng/m^3), whereas the minimum values were registered at Tarragona ($\Sigma TM_{2.5}$ 103 ng/m^3) and the Canaries ($\Sigma TM_{2.5}$ 84 ng/m^3) sites (Moreno et al., 2006a). In detail, however, these average ΣTM figures disguise large variations in specific elements as clearly shown in Tables 5 and 6.

TABLE 5. Average annual concentration f heavy metals in six sites # in Spain.

Metal	1		2		3		4		5		6	
	A	B	A	B	A	B	A	B	A	B	A	B
Ti	30.2	10.6	22.5	6.1	52.3	12.3	82.9	26.3	59.7	21.8	24.2	8.6
V	3.6	2.3	7.7	5.3	7.5	6.1	15.3	9.5	6.8	3.6	8.3	7.0
Cr	2.5	1.1	2.9	2.2	1.9	1.0	8.2	2.9	1.9	0.9	24.6	15.8
Mn	9.7	4.3	9.2	2.8	11.0	3.2	22.8	9.6	10.7	3.2	86.5	39.2
Ni	2.3	2.2	4.2	3.5	3.8	3.7	7.3	5.2	3.3	3.0	33.0	20.8
Cu	28.1	23.7	32.9	31.7	22.9	17.3	48.5	31.7	10.1	32.9	32.6	29.1
Zn	95.7	34.9	35.0	19.3	14.5	14.0	97.3	55.5	50.5	42.6	417.4	239
As	0.6	0.5	0.8	0.5	0.3	0.3	1.5	1.1	5.4	4.2	1.8	1.5
Se	0.4	0.4	0.5	0.2	0.3	0.3	1.1	0.8	1.5	1.1	2.8	2.8
Sr	4.0	1.4	4.8	1.2	6.5	1.6	6.2	1.9	4.4	1.0	2.9	0.9
Mo	3.9	2.9	2.2	2.6	2.2	0.7	4.0	1.7	1.9	1.4	15.5	12.3
Cd	0.3	0.3	0.3	0.2	0.2	0.1	0.7	0.6	0.8	0.8	1.2	1.0
Sn	1.5	1.3	1.7	0.9	0.5	0.5	4.4	3.7	2.2	1.9	37.5	37.0
Sb	8.2	2.8	6.9	2.6	7.3	0.8	10.8	4.3	2.7	1.0	3.6	1.2
Ba	23.4	4.6	12.1	3.2	17.8	5.6	40.8	9.3	15.8	2.5	14.1	7.2
Pb	22.2	19.0	25.5	17.5	15.3	12.5	57.1	40.3	36.8	26.9	101.7	76.5

Sites: **1**) Alcobendas; **2**) Tarragona; **3**) Canaries; **4**) Barcelona; **5**) Huelva; **6**) Liodio

A: PM_{10}; B: $PM_{2.5}$

TABLE 6. Comparison between concentrations of selected trace metals at six sites # grouped according to their potential health concern, varying from most (Pb+As+Cd) to least (Ti+Ba+Sr) toxic. Average annual levels of PM_{10}, $PM_{2.5}$ and $PM_{2.5}/PM_{10}$ ratio (*) for the three metal groups are shown.

Average	**1**	**2**	**3**	**4**	**5**	**6**
PM_{10} (μg/m^3)	31.9	37.4	37.3	41.0	37.5	31.3
Pb-As-Cd	23	26	16	59	43	105
Ni-Cu-Zn	126	72	41	153	124	483
Ti-Ba-Sr	57	39	77	130	80	41
$PM_{2.5}$ (μg/m^3)	23.3 0.79*	22.2 0.59*	18.6 0.50*	27.7 0.67*	19.3 0.51*	24.5 0.78*
Pb-As-Cd (ng/m^3)	20 0.87*	18 0.69*	13 0.81*	42 0.71*	32 0.74*	24.5 0.78*
Ni-Cu-Zn (ng/m^3)	61 0.48*	55 0.76*	35 0.85*	92 0.60*	79 0.64*	289 0.60
Ti-Ba-Sr (ng/m^3)	17 0.30*	11 0.28*	20 0.26*	37 0.28*	26 0.32*	17 0.42*

Sites: **1** Alcobendas; **2** Tarragona; **3** Canaries; **4** Barcelona; **5** Huelva; **6** Liodio

Migon et al. (2008) reported that lead concentrations in the atmospheric aerosol were monitored in 1986–1987, 1992–1993, 1995, 1997–1998 and 2003–2005 at a North-Western Mediterranean coastal sampling station. After the implementation of antipollution policies on automotive Pb in the second half of the 1980s, Pb concentrations were markedly lowered and then the decrease slowed down, at the latest in 2003, resulting in average concentrations varying between 6.6 and 5.8 ngm^{-3} in 2004 and 2005, respectively. Moreover, a study was carried out in Isfahan (Iran) to estimate the levels of heavy metals in ambient air and Table 7 lists the results of this study.

TABLE 7. Heavy metals concentrations in ambient air of Isfahan in different seasons (ng/m^3).

Heavy metals	Winter	Spring	Summer	Avg.	Min	Max
Cu	1,750	180	380	770	20	2,250
Cr	120	80	32	80	10	160
Cd	15	10	20	16	5	50
Zn	890	360	450	560	40	860
Pb	780	490	560	620	20	1,600

The US Agency for Toxic Substances and Disease Registry (ATSDR) lists the top 20 hazardous substances according to a combination of their frequency, toxicity, and potential for human exposure. In this list As, Pb, Hg and Cd appear as the most toxic metals for human health, being first, second, third and eighth in the list. The EU (Directives 2004/107/EC and 199/30/CE) has correspondingly established annual ‘‘target value’’ desirable threshold limits on concentrations of As (6 ng/m^3), Cd (5 ng/m^3).

6. Exposure Assessment

Exposure assessment is the determination of the emissions, pathways and rates of movement of a substance and its transformation or degradation in order to estimate the concentration/dose to which human populations or environmental compartments are or among be exposed. In general, exposure may be defined in the first instance as the instantaneous *intensity* of an agent that is relevant to a particular adverse health outcome at an appropriate interface between the environment and the exposed individual. In this definition, the intensity is reflected in the metric that is chosen to measure the exposure (Van Leeuwen and Vermeire, 2007).

Heavy metals can be transported with differing levels of efficiency to the target host depending upon the transport pathways. Exposure may occur directly by ingestion, inhalation, or dermal contact. The relative contribution of different pathways must be assessed by examining the nature of human activities which may be expected in particular exposure settings. This evaluation will identify both the situations for which the greatest exposure may be anticipated (young children ingesting soil while at play, for instance) and the safety standards that will eventually be needed. Again, the actual concentration of toxic chemical in the host cell depends partly on assessments of host-environment contact and partly on knowledge of absorption and metabolism of the particular chemicals.

Assessment of exposures to air pollutants refers to the analysis of various processes that lead to human contact with pollutants after release into the environment. The routes of exposure to air pollutants include not only inhalation, but also ingestion and dermal contact with pollutants that have deposited or settled on soil, surfaces, foods and other objects (Figure 3). Exposure assessment requires the integration of environmental quality data with an estimate of the rate of human contact with contaminated media. This aspect of risk assessment should rely heavily on local data, since it allows an assessment of how particular

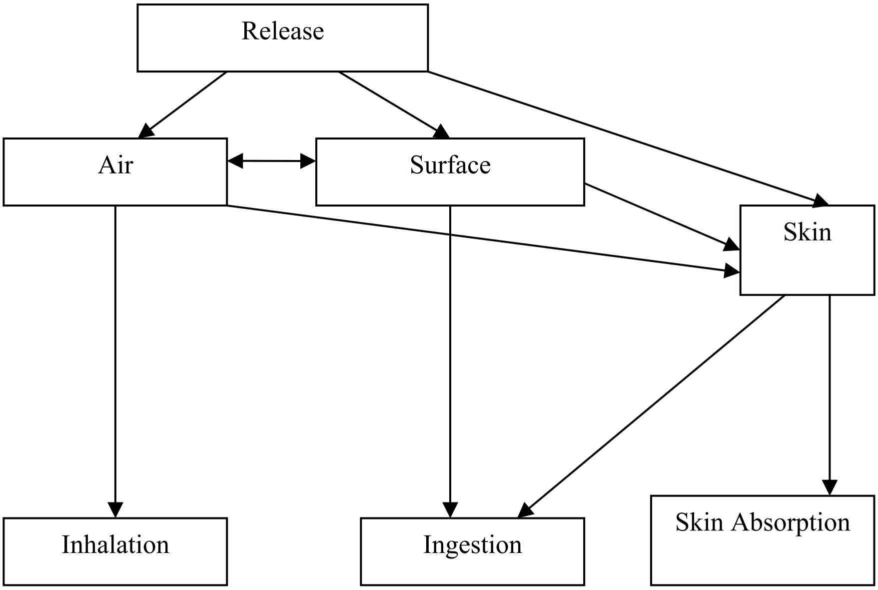


Figure 3. Pathways showing how occupational and environmental releases can result in human exposure to contaminants.

local conditions and cultural practices affect risk potential. In this chapter we focus on the air inhalation pathway only, although ingestion or dermal exposure routes may be quite relevant for some pollutants, such as lead, which contaminate either settled dust or residential indoor surfaces

Exposure assessment studies in the developing world are important. Although recent years have seen an increasing number of industry related pollution exposure studies, exposure assessment data on this topic are still limited. Differences among measuring methods and a lack of strict quality control in carrying out exposure assessment make it difficult to generalize and compare findings between studies.

Air sampling (or monitoring) is one part of an overall process called *exposure assessment*, which is aimed at defining an individual's or a group's exposure to chemical, physical, and biological agents in the environment. The source of the agents may be natural, industrial operations, vehicle emissions, homes, agriculture, demolition operations, waste disposal sites, accidental releases, intentional releases from terrorism or similar events, or others. The population whose exposure is being measured may be employees of the organization.

7. Modeling Personal Exposures

Pollutants from outdoor sources can also penetrate indoors, and indoor micro-environments may be a significant locus of exposure to outdoor pollutants. One key step in exposure assessment is, therefore, to predict (i.e. model) or directly measure the geographic and time profile of the pollution concentrations in the study region of interest.

Various air pollution dispersion models- for example, the EPA's Industrial Source Complex-Short Term (ISCST) and mobile source model Mobile 5 - can be used to predict concentrations of air pollutants both in urban and suburban environments using information on characteristics of the sources in the area and the associated emission rates of the pollutants. These models allow determination of fate and transport of the pollutants in the environment and provide concentration values at different receptor points, i.e. locations, in the assessment area.

In the micro-environmental model of exposure, personal exposures (E) are estimated by the combination of pollutant concentrations in selected microenvironments *(Ci),* with the fraction of time spent in each of these micro-environments (f_i):

$$E = \sum_{i=1}^{n} f_i C_i \tag{1}$$

For air pollution, most exposure models utilizing the data gathered from these field investigations have focused on the inhalation pathway and two key microenvironments: indoors at home, and outdoors. A variety of physical and statistical modeling methods have been developed for predicting exposures to gases and particles (Duan, 1982; Spengler and Soczek, 1984; Ott, 1985; Lioy, 1990; Ryan, 1991). As described by Eqs. (1 and 2), in the physical models framework, total personal exposure is modeled as a sum of exposures encountered in various microenvironments. Numerically, daily personal exposures *(E i)* of an individual i are computed as the sum of time-activity weighted micro-environmental exposures:

$$E_i = \sum_{j=i}^{m} E_{ij} = \sum_{j=i}^{m} f_{ij} \times C_{ij} \tag{2}$$

where $\boldsymbol{E_{ij}}$ is the exposure to individual "${}_i$" in microenvironment ${}_j$(µg/m^3), f_{ij} is the fraction of time spent by person "" in microenvironment "${}_j$" during the 24-h prediction period *(i.e. ti$_j$/24),* "C_{ij}" is the average pollutant concentration (µg/m^3) in microenvironment "${}_j$" when individual "${}_i$" is present, and "${}_m$" is the number of microenvironments considered in the model. As mentioned earlier, key assumptions in this micro-environmental modeling approach are that: (1) concentrations of pollutants are distributed uniformly in each of the micro-environments; (2) f_i and C_i are not correlated; and (3) a limited number of microenvironments are sufficient to characterize total personal exposures.

The validity and the precision of the micro-environmental exposure models depend on the number of different microenvironments that are needed to capture most of the variations in the concentrations affecting exposures. In addition, various exposure scenarios within these microenvironments or sub microenvironments also need to be considered.

8. Initiatives to Reduce Air Pollution and Prevention Policies

There exists a wide scope of mitigation options and strategies for air pollution abatement. The feasibility of these options varies from one country to another depending on the social and economic welfare of each nation. However, options like setting air quality standards, establishing air monitoring networks, increasing awareness among citizens as well as decision makers, and allocating sufficient funding could be adopted in most Southern Mediterranean countries. Each country has conducted steps and set regulations aiming at solving the air quality problem.

In what follows a brief overview of selected action programs taken to reduce emission of air pollution in general and HMs in particular. There is a continuous reduction of heavy metal emissions in Europe during the last 40 years. Better knowledge of heavy metal sources, emissions, pathways, and fate in the environment and progress in developing efficient emission control equipment has resulted in more efficient regulatory efforts to curb heavy metal emissions from anthropogenic sources very substantially.

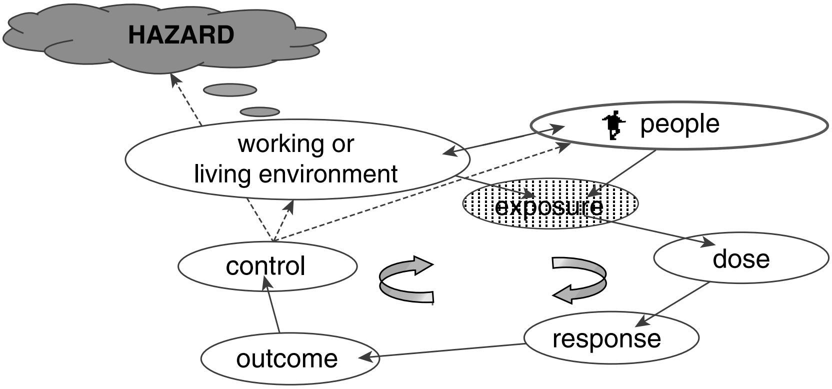


Figure 4. General overview to indicate the linkages between hazards in the environment, human exposure, dose, response, health outcome and control options.

First major decrease of As and Cd emissions in Europe has occurred in the mid-1970s when more efficient electrostatic precipitators (ESPs) and fabric filters (FFs) were employed in Europe to reduce dust emissions from major point sources of emissions, such as smelters, power plants, and cement kilns. The second decrease of As and Cd was observed in the mid-1980s were efficient flue gas desulfurization (FGD) installations were introduced particularly in Western Europe. Finally, the major decline of As and Cd emissions in Europe was observed between 1990 and 2000. This decrease was caused mainly by (1) the implementation of the FGD equipment in large power plants and the other emission controls in other industrial sectors and (2) decline of economy in Eastern and Central Europe due to the switch of economies in these countries (Pacyna et al., 2007).

In high-concern areas, such as found in populated areas, further measures have to be taken to avoid health risks for people living in these areas. Figure 4 shows a general overview to indicate the linkage between hazard in environment and human. The measures already taken to reduce emissions from the industrial site will help to avoid further increases in heavy metal concentrations. In areas with exceeded action limits, measures have to be taken as required by law. Detailed risk assessments could help to take necessary actions to protect public health in this area. The public should be informed about the potential hazards of eating plants grown in that area. Educational programs for schools, informing children about the contamination, should lead to a better understanding of environmental problems and a more sustainable behavior in the future.

9. Conclusions

Urban air pollution and its impact on urban air quality are a worldwide problem. This manifests itself differently in different regions, depending upon economic, political, and technological development, upon the climate and topography, and last-but not least-upon the nature and quality of the available energy sources. Concern about atmospheric pollutants underlies the efforts to establish control programmes in many countries. Policies may be both source-oriented (e.g. technology-based emission management) and effect-oriented (e.g. risk assessment). In most countries, various regulatory instruments are combined into a coordinated control programme. In practice, controlling (anthropogenic) air pollutants is a very complex problem: sources and emissions have to be identified, analytical methods have to be evaluated, risks have to be assessed, critical emissions have to be controlled, and economical aspects have to be integrated.

The situation in developing countries is mixed, however, and in some economies in transition (including Eastern Europe) traffic is becoming the problem. This is a challenge to city planning in these countries, where the long repressed wishes for private automobiles are difficult to reconcile with environmental protection. This is a significant problem because not only are emissions from these units uncontrolled but also the poorest and cheapest types of fuel and even wastes are burnt. Further reductions of emissions from residential units are possible through substitution of these units by central heating plants burning natural and biogas. More extensive introduction of renewable energy sources in Europe may result in reducing the combustion of coal. There are also some ways to reduce emissions of heavy metals to the atmosphere in Mediterranean countries from their major anthropogenic sources through: (1) review development of regulation, policies, and institutional capabilities and its impact on sustainable development in the Southern countries of the Mediterranean basin; and (2) identify the gaps and assess priorities of actions for further developments of efforts for air quality control.

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THE PROBLEM OF HEAVY METAL POLLUTION IN THE REPUBLIC OF ARMENIA: OVERVIEW AND STRATEGIES OF BALANCING SOCIOECONOMIC AND ECOLOGICAL DEVELOPMENT

YURI SUVARYAN, VARDAN SARGSYAN
AND ARMAN SARGSYAN*
Faculty of Economics, Yerevan State University of Economics
Nalbandyan St., House 128, Yerevan 0025, Armenia

Abstract. The presence of heavy metals in environment has been a subject of great concern due to their non-biodegradable nature. It is therefore important to continuously carry out environmental impact assessment and audit in order to evaluate the effects of these metals on the environment and meanwhile to design balanced and sustainable schemes of local industry development. The paper presents overview of heavy metal pollution in Armenia, especially in the regions, where mining industry recently is developed and where indices of heavy metal pollution are very high. Mining industry is the main source of heavy metal pollution in Armenia. Is developed a strategy of modeling of socio-economical and ecological balance of heavy metal impact and a concept of integrated model is presented.

Keywords: Heavy metals, pollution, mining industry, modeling

1. Background

Contamination with heavy metals comes from localized sources mostly from industries, agriculture, sewage, combustion of fossil fuels and road traffic. Heavy metals such as Zn, Cu, Pb, Cd, Mn, etc. are prominent components of industrial effluents, which are discharged into the environment and consequently pollute the aquatic ecosystem. The National strategy of sustainable

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* To whom correspondence should be addressed: arman@asue.am

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development of the Republic of Armenia in the twenty-first century presupposes a planned, stable socio-economic growth of the country, taking into account the reasonable use of nature resources and conservation of the favorable environment for present and future human generations. The transition to a sustainable development should in the long run solve the problem of parity between the socioeconomic and ecological development, elevation of human life quality. Contamination of the environment by ions of heavy metals can bring forth the most unfavorable consequences. There are several investigations around heavy metal pollution in Armenia, which shows the remarkable level of heavy metal pollution in soil, atmosphere and watersheds and also the influence of heavy metals on the health of adults and children.

2. Overview of Heavy Metal Pollution in Armenia

Ions of lead and cadmium cause decreases of crop yields, changes of plants chemical content, accumulation of cadmium and lead. Lead content in atmospheric air of Yerevan makes 1.2–1.3 of Maximum Allowable Concentration (MAC), while at busy high-ways it reaches 16–19 MAC (Jugaryan, 2000). In the atmospheric air of Vanadzor city the concentration of lead exceeded MAC 15–20 times, while in Alaverdi town exceeding was 10-fold (Jugaryan, 2000; Deroyan, 1957). In soils at the territory of Alaverdi mining metallurgical combine and at a distance of up to 2 km aside from this combine the content of lead exceeded MAC 20–40 times, at a distance of 3–5 km exceeding made 10–15 times (Jugaryan, 2000). According to some sources, soils from the vicinity of this combine lead content exceeded the norm 81–109 times (Unanyan, 1987). There are a number of scientific publications on the content of heavy metals, in particular lead, cadmium and mercury in plants and food stuffs (Avakyan et al., 1984, 1987; Evoyan, 1974; Grigoryan and Galstyan, 1980). In plants from polluted areas in the vicinity of Alaverdi combine the content of lead exceeded MAC 25 times, in apples – 15 times, in peaches 2–5 times. In grapes and peaches grown near the Ararat cement plant lead concentration there was 7–9 fold exceeding, in tomatoes, pepper and aubergine - 2.5–3 times.

Lead exposure is a significant but largely unexamined public health issue. The first comprehensive blood lead level (BLL) survey in the Republic of Armenia was performed in 1996 (Saryan et al., 1996). Over 300 venipuncture blood samples obtained from adult workers at four establishments, and from children living in various districts of Yerevan, have been analyzed for lead content. Armenian children studied between 1992 and 1995 were shown to have low to moderate lead absorption (mean ± SD: 6.5 ± 3.4 mcg/dL, highest value 18 mcg/dL, n = 198 samples). None of the Armenian children fell into the CDC “medical evaluation” range (≥20 mcg/dL).

Questionnaire and interview performed to assess level of knowledge on lead of parents of children and the workers, exposed to lead via life-conditions or at work place, as well as teachers and medical personnel has shown low level of their awareness (Babayan et al., 2004). The analysis of data has shown positive correlation between presence of a factory worker in a family and the level of lead in blood of children. Correlation of socioeconomic status and a level of knowledge about lead is revealed.

In 1986 the coefficients of cadmium content in solid wastes (slag and dust flow) of Alaverdi mining and metallurgy combine made 923–7,692. In soils at a distance less than 2 km from the combine cadmium content exceeded the control levels 2–5 times; at a distance of 3–5 km 2-fold, at 7–8 km – 1.1 to 1.7 times (Avakyan et al., 1987). Cadmium content in plants exceeded the MAC 50-fold. In husbandries of the zone of severe contamination (Alaverdi combine) the crop yield in 1983–1987 made 25–30% of control; while in a zone of moderate pollution it was 80% (Avakyan et al., 1984, 1987).

3. Mining Industry in Armenia and Heavy Metal Pollution

Nowadays heavy metal pollution is a key problem for the Republic of Armenia. Armenia is situated in the South of the Caucasus and occupies only 29.8 km^2. The total land in Armenia is 2,974,300 ha. Soil and water pollution by heavy metals is a concern in urban and industrial areas. Mining industry is the main source of heavy metal pollution in Armenia. Copper and gold mining operations in the republic were heavily polluting soils. In Armenia about 30,000 ha of land is polluted by copper, lead and molybdenum due to mining operations in Northeast and South of the republic. The volume of accumulated industrial wastes reaches several hundred millions cubic meters. Land surrounding the Alaverdi copper-molybdenum plant, in radius of 3 km is polluted by heavy metals 20–40 times above norms. Similar enterprises are located in Kadjaran, Kapan, Megri and Agarak, and their surroundings are also polluted by heavy metals (statistical data are not available).

Total 26 polymetallic mines are being developed in Armenia. Among them there are truly giants such as Kajaran Copper-Molybdenum Mine. It is known that its borders stretch so far that whole Kajaran Town is located above rich copper and molybdenum reserves. Next to Kajaran Copper-Molybdenum Mine is the Shahumyan gold-polymetallic deposit, Kapan central deposit. Because of the negative impact of such a number of mining and industrial objects in the whole region of Syunik, including Kapan Town, Kajaran, and Agarak Town, adjacent communities are in a really hard condition close to ecological and social disaster. In some samples taken from the soil, water and agricultural products the content of heavy and toxic metals, such as copper, molybdenum, mercury, arsenic, vanadium, cadmium, selenium etc exceeds MPC (maximum permissible concentration) by ten times, sometimes, by hundreds times.

An important issue for Armenia is the problem of optimal management of water resources and prevention of watersheds from heavy metal pollution. Water resources play important role in the economic development of Armenia. Because of the economic crisis and inoperability of industrial enterprises were seem improvement of surface water quality. Pollution of surface waters, not complete management of water resources are really problems in the field of water resources management in Armenia.

The pollution of the Armenian rivers is conditioned by two main factors. This is the absence of purifying system for municipal sewer, including in big towns, and pollution with industrial flows and ore waters. According to the State Statistical Service, the discharge of sewage in 2008 made up 205 million cubic meters (www.armstat.am).

One of the most polluted rivers is considered to be Voghji transboundary river (southern boundary with Iran) exposed by big enterprises, Kapan Ore Processing Combine and Zangezour Copper and Molybdenum Combine. The breakdown emissions of the Artsvanik tailing, the ore water from Shahumyan and Qajaran Mines also are discharged into this river. On average, the samples taken from the Voghji River exceed the MPC as follows: ammonia – from 3 to 4.6 times, sulphate ions – from 1.6 to 2.5 times, aluminum – from 1.6 to 8 times, vanadium – 3 times, manganese – from 3 to 3.5 times, copper – 6–7 times. The Akhtala River, a tributary to the Debed transboundary river (the northern border with Georgia), is also a highly polluted river. The drainage of the Akhtala Ore Processing Factory flows into this river. According to the data submitted by the Monitoring Center of the Ministry of Nature Protection of RA (www.mnp.am), in the ore water of the Akhtala River the content of copper, zinc and manganese exceeded MPC by 9,199.0–10,656, 363.1–397.6 and 580.9–642.3 times, respectively. One of the main sources for the river pollution is the Akhtala tailing which is already overfilled and doesn’t fulfill the functions of a hydraulic cleaner. The Debed river watershed basin is one of the important economical centers of Armenia, as is characterized with rich natural resourses, and stands out with developed industry and agriculture. All of these cause serious ecological and environmental problems, especially when the waste management mainly absents in the area of watershed basin.

Investigations of the level of pollution with heavy metals and possible risks related to Debed river watershed basin is one of the important ecological issues in Armenia. Around the problem was made research for the period from 2004 to 2008 with the aim to discover the matter of pollution with heavy metals from big residential areas of the watershed basin and the clean-self possibilities of waters of the rivers (Danielyan, 2008). On 2005 in the waters of the river

Pambak exceeds of MPC for culture-municipal water use are observed from toxic metals for the iron (MPC-0.5 mg/l) and for the copper (MPC-0.01 mg/l). On 2006 in the river Pambak the exceeds of MPC have been mentioned also for the manganese (MPC-0.1 mg/l), aluminum (MPC-0.5 mg/l), lead (MPC-0.03 mg/l), zinc (MPC-1 mg/l) and for cadmium (MPC-0.01 mg/l). On 2006 compared with 2005 were observed remarkable increases of heavy metals' concentrations in waters of the river Pambak. In waters of the river Debed exceeds of MPC have been mentioned for the iron, cooper, manganese, aluminum and for the zinc. On 2006 exceeds of MPC have been mentioned also for the lead. It is distinctive that concentration of cooper has exceeded the MPC in the river Debed, where is developed the metallurgical industry in area of the watershed basin.

4. Modeling and Optimal Control

The problem is focused on the establishment sustainable mining strategies in Armenia. Mining is one of the main branches of industry in post-soviet Armenia. Armenia nowadays has resource based economy and mining industry generates notable part of GDP in Armenia. From the other hand mining regions mostly are polluted with heavy metals, what has its social consequences. Solutions for secure policymaking must be obtained under one "social-ecological-economical" system. In order to balance economic development with environmental and social sustainability it is necessary to realize multifaceted analysis. Sustainability concerns not only economic, but also social systems and it is extremely important not to separate social processes from economic activities. Integration of economic, ecological and political values into decision making is the mail condition. According to above mentioned, in order do develop environmentally sustainable strategies and realize optimal control of heavy metal pollution, social and economical factors must be considered in the same framework.

A concept of a model system (integrated model) for obtaining optimal strategies of development of mining industry in Armenia was developed (Figure 1). The main idea of the concept of integrated model is the definition of influence of economic activities on child morbidity and working out of socially optimal economical strategies in mining regions of Armenia. Social influence of economic development could be calculated directly. Such classic approach is being widely used in modeling practice. There is a new approach in the presented model, which is based on the definition of intermediate assessment and is presented with the help of "built-in" function. The model integrates social, ecological and economical factors of heavy metal pollution and includes three phases-assessment, intermediary assessment and optimal control.

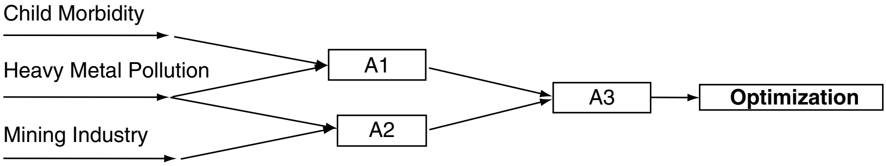

Figure 1. Concept of a Model System for Definition of Optimal Strategies of Development of Mining Industry.

Assessment phase consists from two econometric models – A1 and A2.

A1 presents environmental assessment of mining industry in corresponding regions and definition of the following pollution function

$$P = f(E) \tag{1}$$

where, P – index of heavy metal pollution, E – mining production index.

A2 presents social assessment of heavy metal pollution and definition of social function:

$$M = z(P) \tag{2}$$

where, M – index of child morbidity, P – index of heavy metal pollution,

The idea of the second phase is social assessment of economic activities thru environmental factor (pollution) and working out of intermediary assessment function based on built-in environmental function.

$$M = s(P),\ M = s[f(E)] \tag{3}$$

Calculation of parallel influence of production index and pollution index on morbidity could be realized by power function model:

$$M = APx1Ex2 \tag{4}$$

where x1 and x2 are elasticity coefficients of pollution and production.

As a methodological base of optimization optimal control model will be more acceptable. Function of optimal control of heavy metal pollution is based on the result, which was developed in the model A3, during the second phase.

5. Concluding Remarks

In order to develop socioeconomical and ecological sustainable solutions, currently there is a need, to investigate, the influence of industrial development, as a main source of heavy metal pollution, on the morbidity. Econometrical investigations showed that there is a definite correlation between heavy metal pollution and indices of child morbidity. A scheme of models was constructed to reveal optimal solutions for development mining industry in Armenia.

Analysis of the situation with heavy metal pollution in Armenia have revealed following conclusions:

- There is a notable level of heavy metal pollution of air soil and especially watersheds in Armenia,
- Heavy metal pollution remarkable influence on the morbidity of adults and children in Yerevan and regions with developed mining industry,
- Municipal and industrial wastes are the main sources of heavy metal pollution in Armenia. Was revealed the influence of municipal and industrial wastes of cities Vanadzor and Alaverdy on formation of heavy metal concentrations of chemical composition of the rivers Pambak and Debed.
- During last 5–6 years were observed remarkable increases of heavy metal concentrations in waters of the main rivers, which could be related with development of the economy in that area.
- Condition of the water environment directly or indirectly reflects to other components of investigated rivers watershed environment, because the close linkages and interactions between them (atmosphere, land and biodiversity).

Overexploitation and pollution of water and degradation of aquatic ecosystems directly affect human well-being too, as human well-being and environmental sustainability are interconnected. Especially in the case of pollution with heavy metals there are real risks related to the agricultural lands and food contamination with heavy metals, to the rise of water treatment cost, related to the rises of chronic diseases.

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HOW TO GROW ENVIRONMENTAL – SOUND BIOFUELS

ALEKSANDRA SAS-NOWOSIELSKA[1]*,
MARTA POGRZEBA[1], ANDRZEJ KITA[2],
EUGENIUSZ MAŁKOWSKI[3]
AND HANNA SAS-NOWOSIELSKA[4]
[1] *Institute for Ecology of Industrial Areas,*
6 Kossutha Str., 40-832 Katowice, Poland
[2] *Department of Analytical Chemistry, University of Silesia,*
9 Szkolna Str., 40-006 Katowice, Poland
[3] *Department of Plant Physiology*
Faculty of Biology and Environmental Protection
University of Silesia,
28 Jagiellonska Street, 40-032 Katowice, Poland
[4] *Department of Plant Anatomy and Cytology, University*
of Silesia, Jagiellońska 28 Str., 40-032 Katowice, Poland

Abstract. EU strategy of renewable energy development assumes an increase of energy from renewable sources, up to 7.5% in 2010 and to 14% in 2020. In terms of that project production and usage of woody and herbaceous energy crops for power and heat generation on contaminated sites is said to be a proposition for wide spectrum of brownfields. Persistent soil pollutants, like heavy metals, are source of soil degradation and create the most complicated problems, as the phenomenon of natural attenuation, which is functioning in case of many other pollutants, is not applicable here. For environmental friendly combustion process (Nussbaumer, 2003) plants used as energy crop, should be free from harmful compounds. There is a luck of easily available information related to the physiological properties of plants concerning heavy metals uptake. Heavy metal concentration in plants is related to the plant species and cultivars. Possibilities of using the list of plants with the low level of heavy metal shoot concentration should help to grow environmentally safe energetic crop. For production of biomass with low level of heavy metals different chemo-stabilization scenarios for various areas are expected. First contaminated soils should be remediated before energy crop production. Chemicals introduced to the soil bind metals and diminish metal uptake by plants. Appropriate soil pH also could stabilize metal migration in the soil compartment.

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* To whom correspondence should be addressed: sas@ietu.katowice.pl

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1. Introduction

A rapid development of industry resulted in enormous soil pollution in the vicinity of industrial sites. Most countries still suffer from a problem of highly contaminated land, which is a result of careless attitude of former management to the environmental issues. The early history of soil pollution is similar in all industrial countries of Europe.

Industrial, agro-technical and human activity, and household effects of natural forces are the causes of adverse changes in soil in many areas of Poland (Table 1). Those degraded areas, in a common view are unsuitable for any particular way of development without proper rehabilitation. The highest soil degradation was caused by opencast mines, steel mills and landfills while chemical degradation occurred mainly around large chemical plants, petrochemical and steel factories, and industrial storage areas for industrial and municipal waste. Specially, the bioavailable fractions of heavy metals are an issue of particular concern as these may easily penetrate most environmental components, including the food chain.

TABLE 1. Devastated and degraded land requiring reclamation and management.

Needs of reclamation in Poland*	1990	1995	2000	2005	2006	2007
Devastated and degraded land [ha]	93,679	72,245	71,473	64,978	65,143	64,373

*Ministry of Agriculture and Rural Development

In the vicinity of a former zinc and lead smelter approximately 11,000, 10,000 and 500 mg/kg of zinc, lead and cadmium in soil respectively, would be expected (Kucharski et al., 2005). Unfortunately, commercially available energy plant species, have rather limited application to post-industrial areas due to their low resistance to the pollution.

Soil cleaning technologies are generally cost consuming and unaffordable for developing countries (Cunningham and Berti, 2000; Laperche, 2001; Sas-Nowosielska et al., 2008b; Kucharski et al., 2008; Simeonov et al., 1999; Simeonova and Simeonov, 2006; Simeonova and Simeonov, 2006a).

The soil techniques allowing chemical stabilization of pollutants, could be very practical, considering their technical simplicity and low cost input. In those techniques the chemicals bind the excess of metals helping also to maintain an appropriate pH and required plant nutrition (Sas-Nowosielska et al., 2007; Kucharski et al., 2005). First, pollutants are bound chemically forming insoluble compounds in soil, then the surface would be stabilize with plants. Concerning this way of soil treatment, among the various stabilization methods the most promising seems to be the addition of zeolites, lignite, phosphates and lime. The beneficial influence of phosphate was already reported by Laperche (2001) and Kucharski et al. (2005). The possibility of using phosphates for zinc immobilization in soils contaminated simultaneously with other metals has also been reported by other authors (McGowen et al., 2001; Hamon et al., 2002).

The species and cultivars/lines are also of great importance concerning their predisposition for heavy metal uptake and level of accumulation in aerial parts (stem and leaves). Metal ions are mobilized by roots by secretion of chelators (organic acids) and acidification in rhizoshere. In roots metals are chelated and excess off them is located in the vacuolas. From roots to the aerial parts, metals in form of hydrated ions and chelates are transported to shoots and leaves where unloading and tissue distribution take place (Clemens et al., 2002).

In Poland, biomass for combustion can be mainly obtained from short rotation willow coppice (Table 2). Plantations of miscant and spartina are also suggested (Budzyński and Bielski, 2004).

TABLE 2. Renewable energy share and estimated area of energy plant cultivation in Poland (Budzyński and Bielski, 2004).

	year	
	2010	2020
Renewable energy share [%]	7.5	14
Area of energy plant cultivation [th. ha]* minimum/maximum	136/170	253/317

*willow plantations

Modernization of the technologies and other applied restructurisation processes applied specially to the steel, petrochemical and chemical industries, had led to the reduction of the anthropogenic pressures on the environment. As a result, the elimination or significant reduction of emissions (including emission of dusts containing heavy metals) was observed. However, it should be noted that contamination of soils is usually permanent, and therefore the purification process take a hundred years. This issue is of great impact for biofuel crops grown on contaminated soils, which can accumulate contaminants in their biomass. In this case remediation technologies applied for soil cleaning are good solution to the problem.

There are many possibilities for remediation of heavy metal contaminated soils but they are not cost-effective. Forms and costs of selected methods vary depending on the country and technology owner, thus costs presented in table below (Table 3) should be treated only as an arranged price.

TABLE 3. Forms and costs of selected methods are presented below.

Technology	Costs ($/t)
Phytoremediation	25–100
Soil Washing	50–150
Acid Leaching	150–400
Stabilization *In Situ*	111–205
Vitrification *In Situ*	300–500
Thermal Desorption	150–500
Electrokinetics	50–300
Landfilling	100–500

Phytoremediation includes several biological methods that utilize properties of specific species of plants to take up and accumulate pollutants from soil McGrath et al., 2002). Those techniques are considered as clean, cost-effective and non-environmentally disruptive technologies. For soils highly contaminated with lead, cadmium and zinc such methods are suggested. One of the phytoremediation methods is phytostabilization. It is based on the property of certain plant species to immobilize contaminants in the soil thought accumulation and absorption by roots, adsorption onto roots' epidermis or precipitation within the root zone. The method was found to be very useful on the discussed areas (Kucharski et al., 2005). However phytostabilization does not remove contaminants from the soil, but reduces the hazards to human health and environment via reduction of bioavailable forms of contaminants.

A drawback of phytoremediation is that it is more a biological than technical approach, and it is difficult to create a definitive protocol that could be applied to any polluted site. The limiting factors differ from site to site, and therefore each project protocol, must be customized to specific conditions of site.

2. Possible Way How to Grow Environmental – Sound Biofuels

The choice between cultivars – as was said plant species and cultivars play a crucial role in heavy metal uptake and level of accumulation in their organs (roots and shoots/leaves). Biomass production for energy purposes needs to be free from contaminants. Evaluation of simultaneous plant exposure to nickel and cadmium (Figure 1) through the root was investigated on five varieties of

Festuca rubra and *F. arundinacea.* Plants were grown on a modified nutrient solution prepared according to Blaylock et al. (1997), where tartar iron was replaced by citrate salt. Cadmium and nickel were added to the nutrient solution in concentrations of 30 µg/l each. Physiological properties of cultivars for cadmium and nickel accumulation in leaves and root were tested (Figures 1–4).

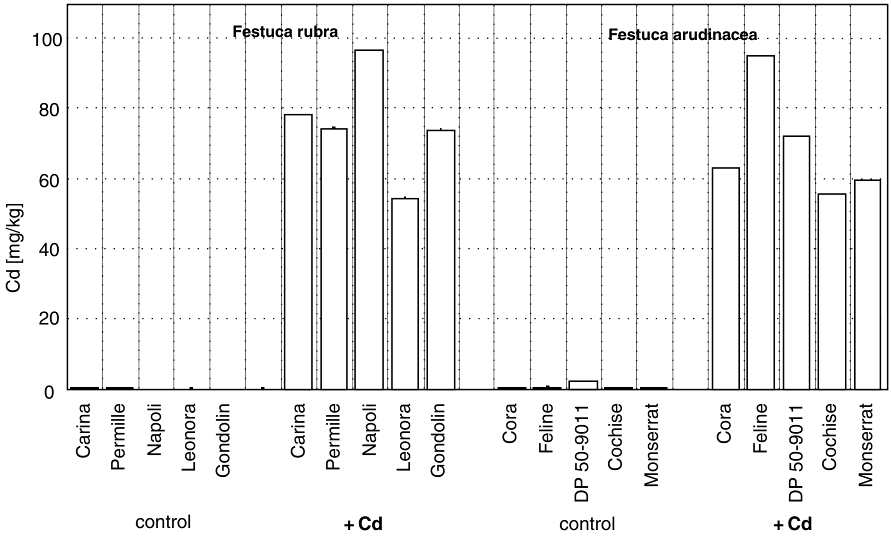


Figure 1. Cadmium in Festuca rubra and arundinacea leaves.

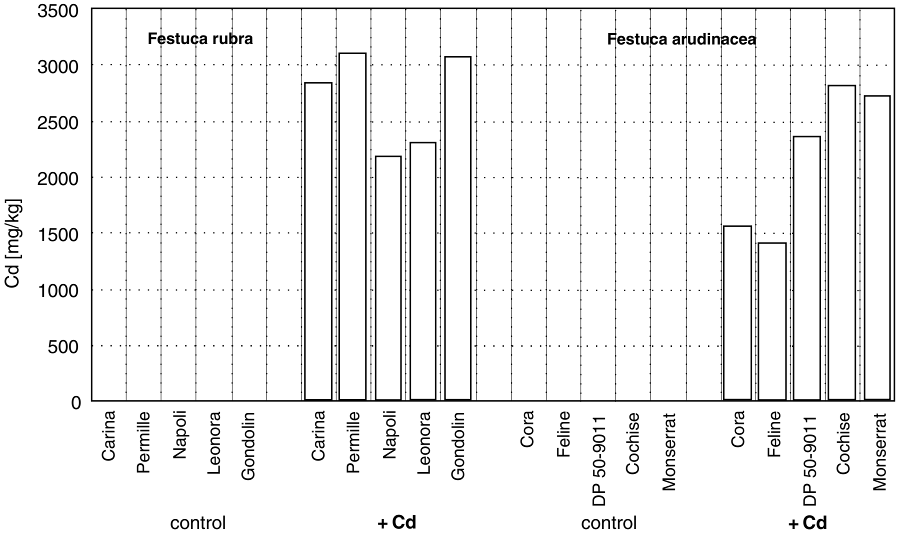


Figure 2. Cadmium in Festuca rubra and arundinacea roots.

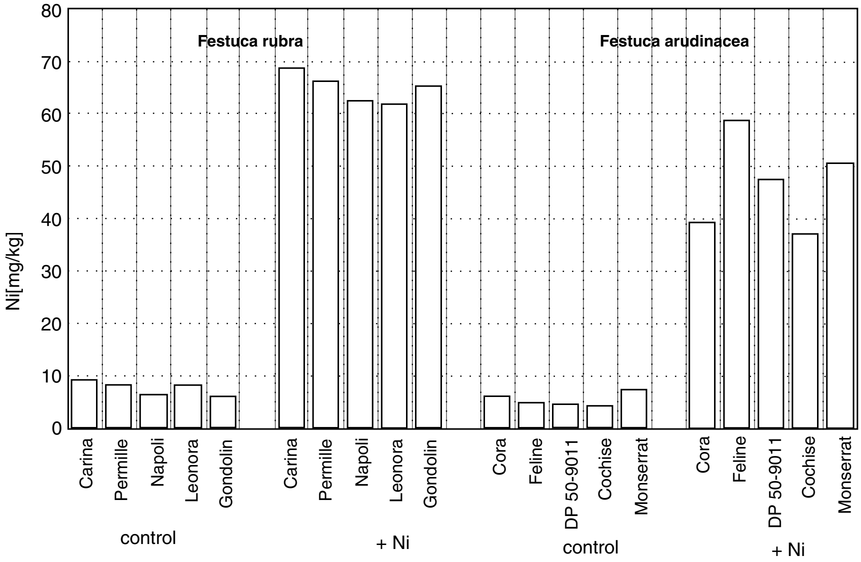


Figure 3. Nickel in Festuca rubra and arundinacea leaves.

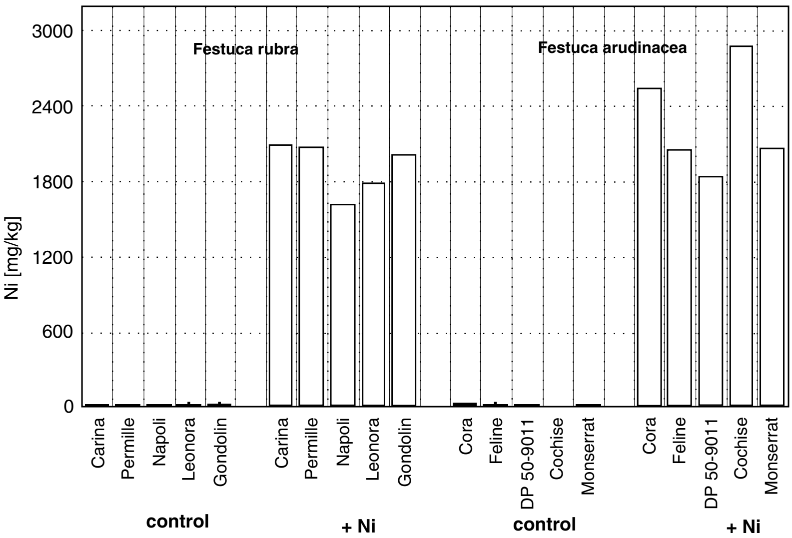
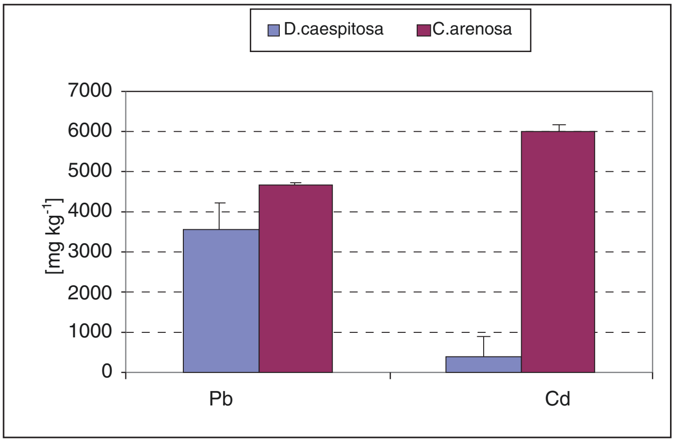


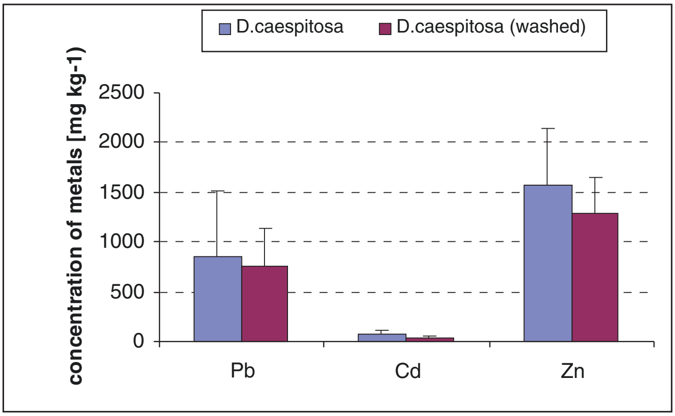
Figure 4. Nickel in Festuca rubra and arundinacea roots.

Evaluation of cadmium concentration in Festuca cultivars clearly showed the differences in accumulation of metals. The point is that for production of biomass as a solid fuel, so called shoot excluders should be taken into consideration. According to presented results *F. rubra* var. *Leonora* and *F. arundinacea* var *Cochise* may be treated as Cd shoot excluders whereas *F. arundinacea* var. *Cora* and *Cochise*, Ni shoot excluders.

Washing processes – airborne particles adhere to the leaf surface affecting the chemical composition of the plant, especially in industrial regions. Evaluation if washing procedures for aerial parts of plant is enough to avoid this problem (Figure 5). Small differences in Pb and Cd concentration between washed and non-washed plant material (e.g. *Deshampsia caespitosa*) were found. That means that the washing procedure was not found as a panacea for diminishing metal content in biomass.



a)



b)

Figure 5. Differences in lead, cadmium and zinc concentration in biomass before (a) and after washing procedure (b).

Maintaining of pH – soil pH affects the availability of nutrients as well as heavy metals in soils. Many heavy metals become more water soluble and more bioavailable under acid conditions. A pH range of 6–7 is said to be generally most suitable for biomass production, but different plant species may have different requirements.

In natural systems the pH is affected by the mineralogy, climate, and weather but because of to physiological acidic fertilizers and/ or acid rains soils may need correction of the pH. Liming is a common method to increase the pH. Dolomite, an incinerated coal mine waste with limited availability, as well as sewage sledges may also increase the pH value (Figure 6).

Possibilities of heavy metal chemostabilization – phytostabilization, which is a part of phytoremediation converts soil contaminants into inert, immobile forms using metal tolerating plants and soil amendments (Salt et al., 1995; Berti et al., 1998). The mechanism may include absorption, adsorption, accumulation, precipitation or physical stabilization of contaminants in the root zone. Plants with well-developed root systems prevent contaminant migration via wind and runoff (Vangronsveld and Cunningham, 1998; Knox et al., 2000, 2001). Phytostabilization may be applicable to large areas of contaminated soil, sludge and sediments that are not amenable to alternative forms of treatment for remediation of heavily polluted sites (Li and Chaney, 1998; Vangronsveld, 1998). The best for fitostabilization are carefully selected indigenous species of grasses, shrubs or trees, which are able to develop a dense and strong root system and produce good amounts of biomass (Lepp and Dickinson, 1998; Li and Chaney, 1998; Vangronsveld and Cunningham, 1998).

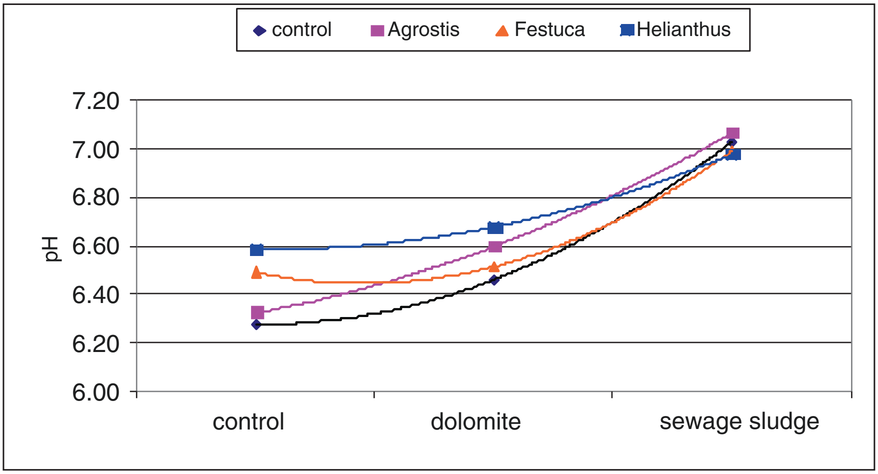


Figure 6. Changes in pH value after dolomite and sewage sludge application to the soil.

Changes in pH value have resulted in lower concentration of Cd and Pb in the soil (Figures 7 and 8). Two- and sevenfold reduction of soil cadmium and 30% of lead was found in leachates.

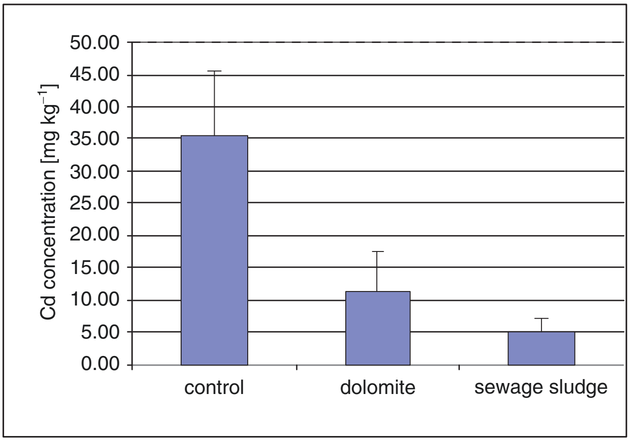


Figure 7. Bioavailable forms of cadmium ($CaCl_2$ extraction) in contaminate soil after dolomite and sewage sludge application.

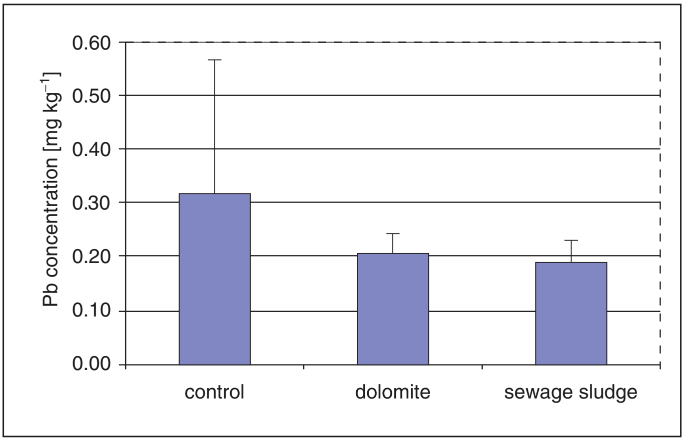


Figure 8. Bioavailable forms of lead ($CaCl_2$ extraction) in contaminate soil after dolomite and sewage sludge application.

As was shown introducing stabilizers (lignite, zeolites) to the semi-contaminated sites lowered content of cadmium in barley straw (Figures 9 and 10). Unfortunately the variety of plants may have bigger impact on Cd shoot content then amendments (Figure 9).

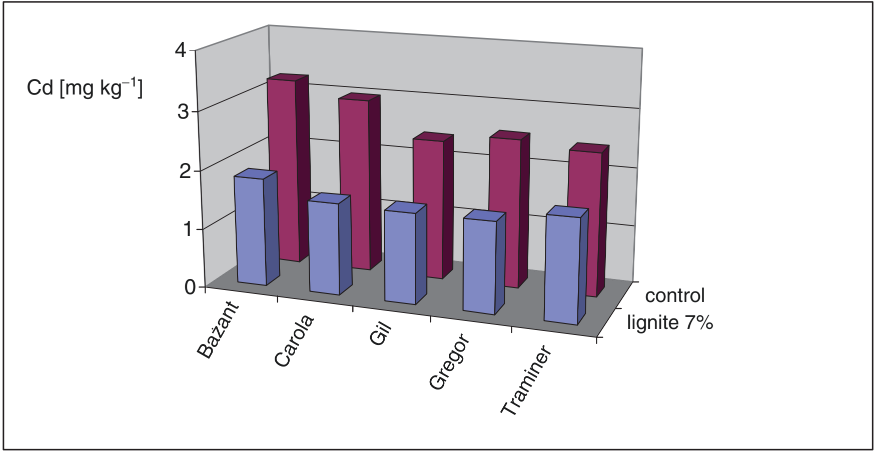


Figure 9. Cd concentration [mg/kg] in straw of barley Mauritia and Nagradowicki cultivars.

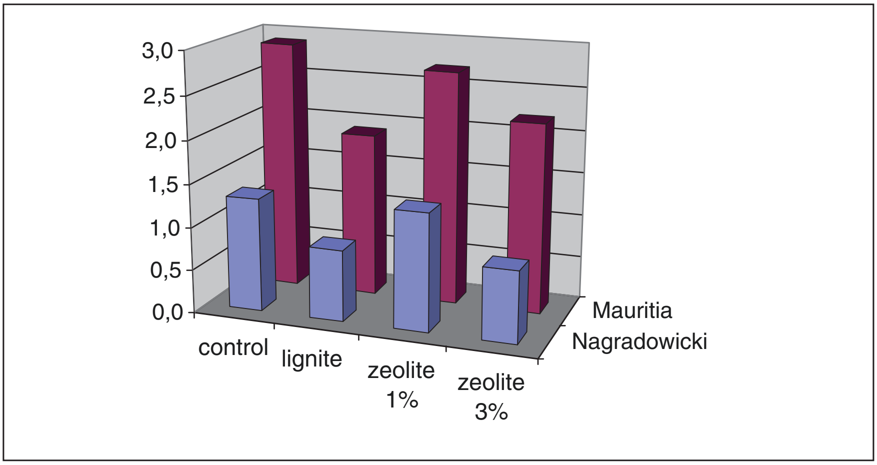


Figure 10. Concentration of cadmium in barley straw after lignite addition to the contaminated soil.

In recent years various modifications of phytostabilization were implemented with good effects to prevent heavy metals migration and water or wind erosion of highly contaminated land (Kucharski et al., 2005; Sas-Nowosielska et al., 2008a, b).

The best for phytostabilization are species, which do not accumulate pollutants in stems and leaves. The issue is raised due to potential possibility of ingestion of contaminated aboveground part of plant by insects or mammals and through this way penetration of the food chain. Contaminated falling leaves blown by the wind may also create the parallel problem.

Good fertilization and watering is a routine in all land reclamation activities, as the plants are forced to grow in very unfavourable environment. The key factor however, is a species of plant used, which has to be highly tolerant to the pollutants contained in remediated soil. In extreme conditions the soil will not support the growth and plants will soon die.

For highly contaminated soils located in the vicinity of former non-ferrous metal smelter in Upper Silesia Region (southern part of Poland) stabilization seems to be the most appropriate method for remediation purposes. Based on analytical results, the soil was classified as loamy, highly polluted with heavy metals (total Pb – 8992 mg kg^{-1}, Zn – 11280 mg kg^{-1}, Cd – 530 mg kg^{-1}). About 73% of Pb, 69% of Zn and 68% of Cd existed in potentially bioavailable form (0.43 N HNO_3 extraction), whereas 0.06, 3.3 and 8% in soil solution (0.01M $Ca(NO_3)_2$ extraction) respectively.

In spite of a very good care taken (fertilizing, watering), control soil did not provide stimulation of growth and development of such plants as: *Agrostis capillaris*, *Salix viminalis*, *Festuca rubra*, *Armoracia lapathifolia* and *Helianthus tuberosus*. Additives as Biodecol and sewage sludge have changed pH and EC of leachates from mesocosms, whereas, sewage sludge has slightly moderated increase of pH and EC.

Analysis of soil leachates has shown no metal binding effects when Biodecol was added to the contaminated soil and an increase of elements (Mg, Na, Ca, Ni, Al) after sludge addition. The general remark was that there was no stabilizing effect detected when tested amendments were applied.

When the soil was amended with SF (superphosphate) and SF with lignite (L) increase of biomass production (Figure11) and decrease of metals in shoots and roots (Table 4) was observed.

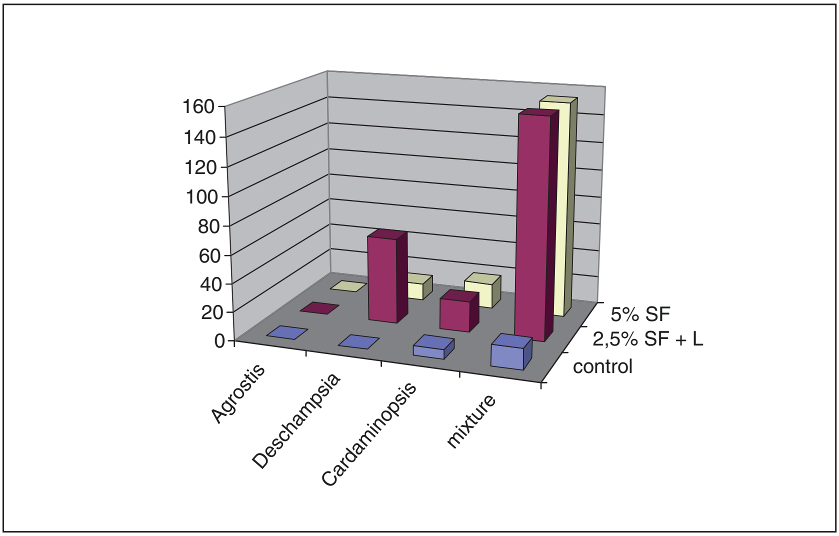


Figure 11. Biomass production (g/pot) after amendment application.

TABLE 4. Effect of amendments on accumulation of Cd, Pb and Zn (mg/kg d.w.) in *Deschampsia caespitosa* plants.

Part of plant	Control			Soil with superphosphate			Soil with lignite and superphosphate		
	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
Shoot	75	715	1,516	16	98	295	12	69	344
Root	889	4,870	5,925	220	868	3,318	201	830	1,873

3. Conclusion Remarks

At highly polluted sites phytostabilization should be carried with the use of crops that can lead to economical benefits of these sites. Appropriate chemical additives, when introduced to the contaminated soil, bind metals and lower metal uptake by plants. Furthermore, low level of metal availability in soil result in increase of environmental-sound biomass production.

Anyway, the impact of future research should be put on developing genetically modified crop species, able to exclude heavy metals from shoots. Apparently such plants should be helpful in remediation of medium contaminated areas.

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NATO ADVANCED RESEARCH WORKSHOP ON ENVIRONMENTAL HEAVY METAL POLLUTION AND EFFECTS ON CHILD MENTAL DEVELOPMENT: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

LUBOMIR SIMEONOV[1]*, MIHAIL KOCHUBOVSKI[2],
BIANA SIMEONOVA[3], CAMELIA DRAGHICI[4],
ELISABETA CHIRILA[5] AND RICHARD CANFIELD[6]
[1] *Solar Terrestrial Influences Institute*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str. Bl.3, 1113 Sofia
Bulgaria
[2] *Institute of Public Health of the FYRepublic of Macedonia*
50 Divizija No. 6, 1000 Skopje,
FYRepublic of Macedonia
[3] *Institute of Electronics, Bulgarian Academy of Science,*
72 Tzarigradsko Shossee Bul, 1784 Sofia, Bulgaria
[4] *Chemistry Department, Transilvania University of Brasov*
29 Eroilor Bldv., 500036 Brasov
Romania
[5] *Chemistry Department, Ovidius University of Constanta,*
124 Mamaia Bldv. 900527 Constanta
Romania
[6] *Division of Nutritional Sciences*
College of Human Ecology, Cornell University
302 MVR Hall, Ithaca, NY 14853, USA

Abstract. Heavy metals can be emitted into environment by both natural and anthropogenic sources, mainly mining and industrial activity. Human exposure occurs through all environmental media. Infants are more susceptible to the adverse effects of exposure. Recently more attention is paid to the mental development of children exposed to heavy metals. The purpose of the Advanced Research Workshop was to evaluate the existing knowledge in this field, to recommend a strategy for countries and regions in risk to protect today's children and future

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* To whom correspondence should be addressed: simeonov2006@abv.bg

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generations from exposure to heavy metals, and to propose the necessary coordinated studies. This paper is a draft of the findings, conclusions and recommendations, formulated as a result of the presentations of the key-speakers; of the work of two round-table discussions: one on the need for studies of the health effects of environmental pollution on children's mental development and the second on prevention strategies; and of the finalizing working groups to summarize the output scientific value of the ARW meeting.

Keywords: Environmental pollution, heavy metals, child, mental development, risk assessment, prevention strategy, discussion and conclusions, recommendations

1. Introduction

The Advanced Research Workshop was expected to contribute to the exchange of experience between the scientists from NATO and Partner countries (www.nato.int/science); to provide information to decision makers; to develop a risk-based program for prioritization of management to reduce environmental exposure to heavy metals; to stimulate national policy to address urgent needs and formulate longer-term strategies for managing environmental pollution from heavy metals in countries and regions in risk.

The main objectives formulated to be:

- To review the principle sources for single and complex mixtures of heavy metal pollutants in the environment.
- To identify the suitable methodology for chemical analyses in the environment and in humans.
- To evaluate the existing methods for measuring mental impairment, including their reliability and validity.
- To recommend a standard testing protocol to be used in future research.
- To assess the future role of environmental heavy metal pollution in countries and regions in risk and its effects on children's neurological development.
- To recommend a prevention strategy for protecting children's health and development.

In order to fulfill the main objectives the scientific program of the Advanced Research Workshop was structured to include lectures from scientists, experts in environmental security, pediatrics, neurology, psychology, and experts in cognitive and behavioral assessment in children, in combination with round table discussions and small working groups in order to formulate conclusions and recommendations. We consider that the conclusions drafted at the workshop are fulfilling the objectives; therefore they are presented below accordingly.

2. Conclusions

2.1. TO REVIEW THE PRINCIPLE SOURCES FOR SINGLE AND COMPLEX MIXTURES OF HEAVY METAL POLLUTANTS IN THE ENVIRONMENT.

The sources for single and complex mixtures of heavy metal pollutants in the environment were presented and discussed and extensively reviewed. Some conclusions are relevant. For Cd, Pb and Hg most significant sources in 2007 are from energy related sources associated with fuel combustion, particularly from public power and heat generating facilities and in industrial facilities. Main emissions sources in 2007, concerning to data from 24 European countries: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Germany, Greece, Finland, France, Hungary, Ireland, Italy, Latvia, Luxembourg, Netherlands, Poland, Portugal, Romania, Sweden, Slovenia, Slovakia, United Kingdom: for Cd it is the energy sector, the production and processing of metals, the waste and waste water treatment; for Hg it is the energy sector (mostly thermal power plants and other combustion facilities), the production and processing metals; for Pb it is the energy sector (mostly thermal power plants and other combustion facilities), the production and processing of metals, and waste and waste water treatment. It is evident that the energy sector, besides other pollutants, is simultaneous responsible by Cd, Hg and Pb emissions, while the coal combustion is still the major source for the heavy metals (except Pb). The vehicular traffic still contributes to 25% lead emissions in the environment, (even in countries which are no longer using leaded gasoline) mostly due to residual lead in fuel, from engine lubricants and parts, and from tire and break wear. The emissions from residential units are still being largely uncontrolled.

2.2. TO IDENTIFY THE SUITABLE METHODOLOGY FOR CHEMICAL ANALYSES IN THE ENVIRONMENT AND IN HUMANS

Methods for measuring and analyzing metals in the environment and in human tissue were reviewed in detail. Key points which emerged from the discussion were the importance of sample acquisition and sample preparation prior to analysis, and the need for quality control in analysis programs. Sophisticated analytical equipment and expertise is available at many different institutions across for example the Central and Eastern European Countries, the Balkan region, but this may not be widely known. It may be useful to make researchers in the region more aware of the availability of equipment as a means of enhancing collaboration. It was agreed that a matrix should be generated illustrating the analytic methods which are appropriate or not for analyses of specific metals/media.

Sampling and sample preparation techniques for environmental and biological materials were reviewed. Analytical methodology for environmental analysis has been discussed. Among current suitable methods and techniques for **trace element determination**, most typical are: atomic absorption spectrometry (AAS), inductively coupled plasma with atomic emission spectrometry (ICP-AES), inductively coupled plasma with mass spectrometry (ICP-MS).

Atomic absorption spectrometry (AAS) is the most widely used method and available and best developed technique. Its advantages are that it is a standard technique, selectivity, robustness, reliability and cost effective performance; and is equally applicable for single element technique as well as for multi-element analysis (2–6 elements).

Inductively coupled plasma with atomic emission spectrometry (ICP-AES) is the best current technique for simultaneous multielement analysis of environmental factors (water, soil, sediments digest) and decomposed food samples. The most suitable biological materials are: blood (B) and urine (U); alternative samples are: hair (H), nail (N), serum (S), deciduous teeth (DT) and isotope determination.

Inductively coupled plasma with mass spectrometry (ICP-MS) is strongly encouraged due to its simultaneous multielement performance, selectivity and excellent sensitivity.

Appropriate analyte biological material-technique combination are: As-U-HGAAS; Cd-U-GFAAS, Cd-B-GFAAS, Hg-B-CVAAS; Hg-H-GVAAS; Hg-U-CVAAS; Pb-B-GFAAS; Se-S-GFAAS; Se-B-GFAAS.

For **speciation analysis** the separation techniques are advisable, liquid chromatography (LC), ion chromatography (IC) gas chromatography (GC) and capillary electrophoresis (CE), especially their hyphenation with different complementary detectors. Mostly recommended are HPLC-AAS, HPLC-AFS, HPLC-ICP-MS, GC-AAS, GC-MS, CE-MS, CE-ICP-MS; also used: HPLC-AES, HPLC-ICP-AES, GC-AES. Ion chromatography IC is U.S. EPA method 218.6 for Cr (VI) determination in water.

All of these techniques are suitable for simultaneous multielement analysis:

Hg (total, metilated), Cr (III, VI), As (total, inorganic III, V), Sn (organic), Se (for nutrition supplements).

It was also emphasized that laboratories should deliver reliable results, based on metrological approach: validation, uncertainty estimation, traceability, certified reference materials, and to ensure quality control during the entire analytical process.

2.3. TO EVALUATE THE EXISTING METHODS FOR MEASURING MENTAL IMPAIRMENT, INCLUDING THEIR RELIABILITY AND VALIDITY. TO ASSESS THE FUTURE ROLE OF ENVIRONMENTAL HEAVY METAL POLLUTION IN COUNTRIES AND REGIONS IN RISK AND ITS EFFECTS ON CHILDREN'S NEUROLOGICAL DEVELOPMENT

Methods dealing with examination of neurodevelopment were touched just fragmentally in relation with autism and autistic spectrum disorders in connection with debatable exposure to mercury and hearing changes in children exposed to arsenic in neurotoxicity context.

There were also mentioned more neurodevelopment problems (attention deficit disorder, attention deficit and hyperactivity disorder, aggressivity that could lead to criminal behaviour) or mental disorders (psychosis, anxiety disorder, depression) that could have a potential relation with toxic metals exposure. To validate this information it would be recommended to use comparable guide lines or scales for evaluation of symptoms and diagnostic.

These methods due to their subjective nature need to be standardized as much as possible to achieve comparable results and size of exposed and reference groups of children need to fulfil standard demands from epidemiology point of view.

Achieved. Participants discussed limitations of methodology for evaluating deficits in cognitive function in children. It is important to use standardized evaluations, for example standardized tests of intellectual function. Tests should also be standardized to the population that is being evaluated (i.e., be translated and validated into appropriate languages). There is a need to ensure that researchers have appropriate training in administering tests to infants and children. It is also important to determine the appropriate age of evaluation. Evaluating the effects of toxic exposures on child development requires longitudinal studies whereas cross-sectional studies could be employed to assess the effect of exposure at specific age points. Correlating neurobehavioral function with toxic exposures must distinguish between cases of chronic exposure over a broad age range and acute exposure at a single point in time. Research designs and age of assessment need to be tailored to the characteristics of exposure. Finally, it is important to design assessments that are maximally sensitive for detecting the specific neurobehavioral functions expected to be damaged by a specific type of exposure. For example, if a chemical is thought to interfere with the actions of a particular neurotransmitter then a neurobehavioral test should be applied that evaluates the functions depending on that neurotransmitter.

It is also important to remediate existing pollutant sources and control future pollutant sources to limit their adverse impact on the health and intellectual capital of the Balkan region. Going forward countries in the Balkan region will move from being concerned about heavily contaminated sites and the health effects on children of very high levels of exposure to concerns about less contaminated sites or lower levels of pollutant emissions such as those which are typical of NATO member countries in Western Europe.

2.4. TO RECOMMEND A STANDARD TESTING PROTOCOL TO BE USED IN FUTURE RESEARCH

Achieved. Participants discussed the need for specifying minimum guidelines for conducting studies of neurodevelopment and neurobehavioral effects in children. These guidelines must cover source identification, tissue sampling protocols, chemical analytic methods, research design, methods of neurobehavioral assessment, and quantitative/statistical methods. It may not be possible to develop a single detailed protocol equally applicable to all studies because objectives, regional issues, and exposure profiles may be specific to particular studies. However, at a minimum, a set of guidelines should be established that will allow for valid and accurate comparison of results across studies and across sites within multi-site studies. This will permit comparison of studies and will ensure a solid set of studies which can be used to conduct weight of evidence (WOE) analyses and as a basis for environmental regulations.

2.5. TO RECOMMEND A PREVENTION STRATEGY FOR PROTECTING CHILDREN'S HEALTH AND DEVELOPMENT

Members of the ARW noted that the communication of research findings on children's environmental health cannot ethically stay within the scientific community. Instead, evidence-based knowledge, along with recommendations for concrete methods of protecting children, must be disseminated in a manner that is consistent with the overarching scientific goal of creating new knowledge and using it to improve the human condition. Recommendations to consider and develop the following measures:

- Strengthening national legal supports;
- Strengthening methodological support; - Protection of the environment;
- Developing investigations (qualitative and quantitative content of heavy metals in the environment, assess exposure of different groups of the population to heavy metals, improved methods/strategies for risk assessment and communication of risk assessment results, elaboration of guidelines for chemicals and chemical exposure); - Increased/improved training of health professionals concerning environmental exposures and effects;
- Enhance education of health issues among the public;

- Qualitative and complex medical assistance;
- Increased international collaboration and use of already established international expertise;
- Collaboration with NGOs and affected populations.

It is established that the mostly unregulated emission of heavy metals and toxic chemicals in the Balkan region and areas of Central and Eastern Europe, over a period of many decades, has resulted in widespread contamination of the physical environment and ongoing exposure of the human population to dangerous elements and compounds. The physical and psychological development of infants and children are at particular risk of life-long health impacts that will place an undue burden on the under-resourced health care systems in this region and limit the intellectual capital of these developing countries of great cultural, economic, and strategic importance. There is an urgent need for research to characterize the sources of past and ongoing pollution to the air, water, and soil and to describe quantitatively the exposure pathways and mechanisms in the people residing in this area. The need is also urgent for research into the impacts of exposure to environmental pollutants on the developing fetus, infant and child. Products of this research can be used to advance scientific knowledge in environmental medicine and to support evidence-based recommendations for addressing one of the most pressing threats to human health this region is likely to face in the foreseeable future. A primary goal of this NATO Advanced Research Workshop (ARW) was to identify needs for research and the collaborative relationships among scientists necessary to conduct projects of world-class quality that will have high impact in legislative settings and be of particular importance to the local populations and regulatory bodies of the affected countries and regions.

After 3 days of scientific presentations and lively roundtable discussions, a set of conclusions were formulated and are here used as a basis for recommendations.

3. Recommendations

3.1. RECOMMENDATION 1 – SYSTEMATICALLY DESCRIBE ACTUAL/POTENTIAL EXPOSURE SOURCES AND THE KNOWN OR SUSPECTED CONTAMINANTS

The workshop participants noted that there exists no comprehensive knowledge of the sources, types, and levels of environmental pollution in the Balkan region. Because current information is fragmented and incomplete, the first recommendation emerging from the workshop is to create a catalogue or database of known or suspected sources of heavy metal pollution. This information source would include the geographic location of the source and the area of dispersion, the heavy metals and other toxins involved the proximity of the contamination to human populations, and the likely routes of exposure for each toxin and location. Special emphasis should be placed on the exposure of pregnant women, infants and children. This would create a resource useful for conducting a risk assessment that would classify the magnitude and the immediacy of the danger posed by each toxic site, and also aid in the identification of study sites that are best suited for addressing particular research questions.

3.2. RECOMMENDATION 2 – LINK EXPOSURES TO SINGLE AND MULTIPLE TOXINS TO NEUROBEHAVIORAL OUTCOMES IN CHILDREN

Knowledge of the effects of high and low-level exposures to many toxins remains incomplete, especially in relation to genetic variation, nutritional status, and socioeconomic differences among children. For example, it is unclear whether there is a safe level for childhood exposure to methyl mercury, arsenic, or cadmium. And although it has become widely accepted that there is no safe level of lead exposure, many questions remain as to the sources of individual susceptibility to that metal. Moreover, almost nothing is known about simultaneous exposure to multiple toxins, a typical circumstance for children in the Balkan region. It was felt that after cataloguing sources of pollution and the types of contaminants involved (see recommendation 1) that one or more natural experiments could be designed and carried out to address polyexposures. For example, it is likely that there are sites for which either lead or cadmium are the sole or sources of toxicity, but that at a third site children might be exposed to lead and cadmium together. Sadly, the extent and diversity of contamination in the Balkans is likely to make such studies feasible.

3.3. RECOMMENDATION 3 – CREATE A MECHANISM FOR COLLABORATION AND COORDINATION OF RESEARCH ACTIVITIES WITHIN THE REGION

Workshop participants noted that a limitation of much research in environmental neurotoxicology has been a lack of consistency in the design and reporting standards among various research projects aimed at answering a particular question. The examples of research on lead and mercury exposure were noted as particular cases in which a lack of coordination among researchers has created an unacceptable degree of confusion about the proper interpretation of findings. This has resulted in a tremendous waste of time and effort in translating research into recommendations for protecting human health. The participating ARW scientists recommend that a mechanism be created to ensure, as much as possible, that studies conducted by different research teams can be easily compared and contrasted to facilitate interpretation.

3.4. RECOMMENDATION 4 – CREATE SHARED RESEARCH PROTOCOL STANDARDS AND GUIDELINES

In service of recommendation 3, it was urged that working groups be established to arrive at a consensus regarding a set of "gold standards" for research design, protocols, and quantitative analysis. In particular, it was thought feasible to ensure that all studies used a common subset of techniques for human population sampling, physical and biological sample collection and analytic procedures, neurobehavioral assessment methods, and quantitative statistical methods.

3.5. RECOMMENDATION 5 – DEVELOP AND EVALUATE METHODS FOR EXTENDING RESEARCH-BASED KNOWLEDGE TO COMMUNITY MEMBERS AND TO POLITICAL AND REGULATORY OFFICIALS

Members of the ARW noted that the communication of research findings on children's environmental health cannot ethically stay within the scientific community. Instead, evidence-based knowledge, along with recommendations for concrete methods of protecting children, must be disseminated in a manner that is consistent with the overarching scientific goal of creating new knowledge and using it to improve the human condition. Although it was not assumed that a single method of communication would be equally effective in all states and regions, still it would be essential to make special attempts to determine guidelines that would provide societies with information and associated recommendations needed to prevent children from being damaged by environmental toxicants. This would include information delivered to parents and to officials responsible for regulatory decision making. One particularly important challenge will be to help parents and officials recognize the health consequences of low-level exposures that do not cause immediately observable clinical symptoms.

3.6. RECOMMENDATION 6 – DEVELOP A PROPOSAL FOR RESEARCH FUNDING THAT REINFORCES COLLABORATIONS ACROSS COUNTRIES AND INSTITUTIONS IN THE REGION

Members of the ARW expressed genuine enthusiasm for conducting collaborative research projects designed to document the effects of toxic exposures to children in the Balkan context. A recommendation arising from that sense of a common goal is to obtain funding, first to allow groups of members representing specific areas of specialization (toxicology, child development, physical chemistry, risk assessment, epidemiology) to meet and more clearly formulate specific research priorities and topics that are of high scientific and practical importance and also are feasible within the cultural and financial context. From these meetings would arise one or more proposals for funding specific research projects that would realize the above-stated goal. Sources of funding that were identified include a NATO grant for joint planning meetings, but also NATO, NIH, EU sources, and private foundations for supporting one or more research projects.

Also noted by several participants was the lack of expertise in environmental medicine within the region. Building research capacity in developing countries is a goal of the Fogarty Institute within the U.S. National Institutes of Health. Combining research funding with capacity building would help the Balkan countries develop their research infrastructure.

4. Final Remarks

This NATO Advanced Research Workshop was a success. Of great importance is that participants representing the Balkan countries very clearly characterized the current environmental situation in their region. During research presentations, roundtables, and informal conversations, environmental problems common to all nations and problems specific to a given nation were identified and discussed. Historically, these problems were shown to have been caused in part by a political desire to produce energy and manufactured goods that would improve the standard of living of people in the region; a lack of information about the dangers posed by the methods of industrial production; and possibly, insufficient access by citizens to the information that would have allowed them to evaluate the health risks for themselves. As illustrated by this conference, however, there is now an obvious and sincere concern among many scientists and physicians in these countries about the need to reduce and eliminate existing threats to human health posed by environmental toxin—especially the health of children, the most vulnerable population. Achieving this goal will require a coordinated effort among researchers, physicians, environmental advocates, economists, and government.

Although some positive steps in this direction have been taken; i.e., closing obsolete or particularly dangerous mines and smelters, the remaining problems are immense. Three main challenges toward future progress in environmental health were articulated. They were (1) a need for research-based information to adequately characterize the nature and magnitude of the environmental contamination and also its effects on human health; (2) a need for public awareness of general environmental health issues and issues specific to a given region; and, as part cause and consequence of the above points, (3) a need for sustained attention to understanding and solving environmental problems by government officials.

4.1. RESEARCH

High quality research-based knowledge is a key to meeting these challenges. This is particularly true because the health effects of chronic low-level toxic poisonings are not generally experienced at the time of the exposure. Moreover, the effects may be subtle (e.g., lead) or greatly delayed in time (e.g., radiation). As a result, it is difficult for the human mind to connect cause and effect. As has been repeatedly demonstrated in the field of public health, in the absence of a convincing cause-effect argument there is room for undue skepticism and even intentional obfuscation of fact. Given the unique historical past of the former Soviet states, the standards of evidence for conclusively linking an environmental cause to an adverse health event are expected to be quite high.

4.2. PUBLIC AWARENESS

ARW participants from the Balkan region noted that existing evidence in the published scientific literature or gathered through research conducted with experimental animals or with human subjects living in very different circumstances or geographically distant lands is not generally perceived as immediately relevant to the local setting. It is likely that increasing public and governmental awareness and concern will require concrete evidence of the adverse effects of local toxins in the local population. This knowledge has not been generated due to a lack of sufficient research capacity (expertise in environmental health research, laboratory infrastructure, strong regional scholarly societies and organizations, student training programs, and funding).

4.3. GOVERNMENTAL ACTION

Although research is essential, regulatory change rarely if ever results from increased knowledge alone. Other motivations are required. For the Balkan situation, additional motivation for improving environmental health comes from a desire by many countries for a closer association with the European Union. Consistent with this, a study in 2005 of children's blood lead levels in relation to proximity to a smelter in Veles, FYR Macedonia, report finding very high blood lead levels in school children. In that report the authors refer to Article 1 Directive 99/30/EC for limit values of SO_2, NO_2, and NO_2, PM and lead (Pb) in the ambient air as providing guidance for setting a goal for allowable blood lead levels in children. The authors' concerns reflect a desire to conform to the EU expectation that member countries will implement all necessary measures to ameliorate such situations.

4.4. SUSTAINED EFFORT

The problems associated with environmental pollution in the Balkan region are many and they continue to grow in number. Conference participants agreed that only a sustained effort to address will produce the desired effect. To that end, the activities of the conference participants illustrated a focused eagerness among a diverse group of scientists to collaborate across national, geographic, and disciplinary boundaries, on an ongoing basis, to carry out the research, the public awareness campaigns, and the formation of strategies to produce regulatory change. The first step toward harnessing the intellectual energy generated by this conference would be to hold two to three smaller and more tightly focused working groups to further articulate a plan for moving forward in the areas of research, dissemination, and policy change.

The road to ameliorating the sources and effects of toxic pollution in the Balkans will be long and difficult. This ARW conference brought together the expertise and generated the momentum necessary for taking some firm steps toward protecting the environment and improving the health of future generations of children in the Balkan region.

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