

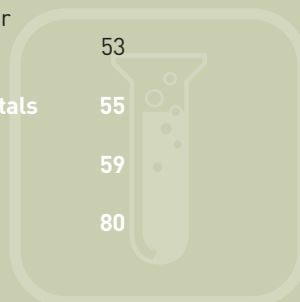
# MERAG

Metals Environmental Risk Assessment Guidance



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Understanding the potential environmental risks posed by metals is a key factor in ensuring that they are produced and used safely. For mining and metals companies this is an important aspect of materials stewardship, whereby companies are extending their interest beyond the plant gate and considering the whole life cycle of their materials. The *Metals Environmental Risk Assessment Guidance (MERAG)* publication presents the most advanced and appropriate scientific concepts for assessing the risk posed by the presence of metals and inorganic metal compounds in the environment.

The MERAG project was launched by ICMM and Eurometaux and recognizing its significance the UK Government – Department for Environment Food and Rural Affairs (Defra) provided official endorsement. In May 2005 Defra hosted a multi-stakeholder workshop which introduced the initiative to regulatory experts and helped gain the support of a number of national authorities including those in Canada, the United States, the European Commission and several EU Member States. This support has already resulted in the use of MERAG concepts in national and regional risk assessment frameworks.

Metals are natural components of the earth and exist in many forms – each with its own specific chemical characteristics that define its interactions with the environment and with living organisms. Traditionally, the majority of risk assessments and consequently guidance materials have focussed on organic chemicals. In many cases such guidance fails to adequately address specific characteristics that must be taken into account to perform accurate risk assessments for metals. Through MERAG we have sought to address this by consolidating the extensive scientific progress that has been made in this field in recent years. The critical concepts are presented in the series of independently reviewed MERAG ‘fact sheets’ which are included on the CD-ROM inside the back cover of this publication. It is hoped that these latest concepts will enable regulators and scientists to create new or adapt local, national or regional risk assessment systems accordingly.

It is our intention that this publication, through periodic updates will continue to provide a solid basis for improvement in risk assessment processes for metals and we encourage all parties to use the material freely (for updates please visit [www.metalsriskassessment.org](http://www.metalsriskassessment.org)). We would welcome any comment on the publication as feedback will enable us to provide further guidance as the science evolves.

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Minerals and metals are naturally occurring substances that are present in a variety of forms (metal species). Understanding the hazards and risks posed by these chemicals, and managing these adequately, have become key aspects of chemicals management policies that are aligned with the principles of sustainable development.

Models and methodologies for evaluating hazards, setting priorities, establishing environmental quality criteria and assessing risks are currently developed and used in several jurisdictions, including the European Union, the United States and Canada. However, because the majority of the compounds assessed are organic substances rather than inorganic metals, the various methodologies and guidance documents provided for performing these assessments are lacking in detail on how to account for metal-specific attributes. Questions related to the specific impact of metals on the environment and on human health and the need to establish ready and accessible metal-specific tools and data sets in order to make informed, science-based decisions have – to an increasing extent – been raised by representatives of local, regional, national and international administrations, as well as by members of non-governmental organizations, private companies and associations.

Several properties of metals and other natural inorganic substances – their natural occurrence, essentiality, homeostatic control mechanisms and acclimatization to diverse natural environments – require specific recognition. Moreover, metals are naturally occurring substances differing in their degree of availability to organisms in both aquatic and terrestrial settings. These differences in 'bioavailability' have often not been properly addressed in the methodologies currently used, but it has become increasingly evident that it is the actual metal species that determines mobility, bioavailability and toxicity of a metal, and that metal speciation depends on the site-specific seasonal and spatial variations existing in a particular water, sediment or soil system (Landner and Reuther, 2004).

Moreover, many base metals are often data-rich, meaning that collecting data and screening and assessing them for environmental or human health risks can be complex, yet it provides comprehensive information to help make science-based decisions. Many governments and regulators have therefore underscored the need to improve concepts and

assessment tools for metals as well as to ensure the availability and accessibility of screened data sets.

This increasing demand for information and knowledge on the properties and behaviour of metals in society and in the environment has prompted numerous research initiatives (notably in Canada, the United States and Europe); as a result, the technical and scientific knowledge on metal-specific aspects has advanced significantly in recent years.

Prominent examples of research fields on metals that have attracted considerable interest over the past 5–10 years are:

- determination of the bioavailable fraction of metal dissolved in water, and development/validation of 'Biotic Ligand Models';
- development of methods and models for the prediction and assessment of trace metal toxicity in sediments and soils;
- assessment of the degree of metal contamination (enhancement of trace metal concentrations, relative to natural background concentrations) in matrices such as sediments and soils;
- description of how trace metals in the aquatic environment are distributed between suspended particles, colloidal material and aqueous solution and of the factors governing their redistribution between phases; and,
- determination of the role of biotic components in the interaction with environmental trace metals (adaptation, acquired tolerance, antagonistic effects).

Given this extensive progress, it was deemed appropriate to develop a guidance document aimed at providing the regulatory community at the regional and international level with the proper scientific and regulatory guidance on the most advanced status of scientific concepts for metals. This Metals Environmental Risk Assessment Guidance therefore consolidates existing experience and recent progress made with environmental risk assessment methods, concepts and methodologies used in chemicals management programs and environmental quality standard setting (soil, water, sediments) for metals.



METALS ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

# PURPOSE AND INTENDED USE

# 01



# 01. Purpose and Intended Use

The principal goal of the Metals Environmental Risk Assessment Guidance (MERAG) is to deliver the basic material to make risk assessments for metals more ecologically relevant – and to provide regulators with material to adapt to their local, national or regional assessment systems. MERAG follows a ‘building blocks’ approach (see section 02) to ensure that the methodologies may also be of use to different jurisdictions and various applications, such as chemicals management assessments and setting environmental quality objectives.

The main target group for the document is professionals in the field of environmental science working with decision-makers at the local, regional, national or international level in both the public and the private sector. While the general chapters could be useful for anyone needing a comprehensive overview of the state-of-the-art of current research on some of the central fields of interest regarding metals in the environment, this document assumes the reader already has a good understanding of the basic principles in risk assessment and the field of environmental quality criteria setting.

Although this guidance document can be used to communicate general principles, tools and recommendations for metals risk assessment, its primary role is as a prescriptive guidance document on how to assess the environmental risks related to metals and metal compounds. The templates and guidance have mainly been built on the experience gained with ongoing or finalized metal risk assessments, but they can be used to improve the realism and reduce the uncertainty of future metal risk assessments or environmental quality criteria setting. In addition, enough flexibility has been built into the system so that it may adapt the principles and methodologies to future legislation and still suit the intended applications.

It is acknowledged that the science surrounding metals continues to be studied intensively and thus is rapidly evolving. Specific principles, tools or recommendations presented in this document and fact sheets may therefore become outdated or may otherwise require modification to reflect the best available science. However, the fact sheets presented on the CD-ROM along with this document will be periodically revised and updated as warranted by advances in regulatory practice and technical understanding.





METALS ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

# SCOPE AND ORGANIZATION

# 02



# 02. Scope and Organization

## 2.1 Scope

MERAG is intended to address the specific properties of metals and other naturally occurring inorganic substances. It summarizes existing knowledge on metal-specific environmental risk assessment approaches and provides comprehensive scientific technical guidance for metals for chemicals management and the setting of environmental quality standards.

Organometallic substances are not included in the scope of the current document. Since organometallic compounds exhibit properties common to both organic substances and inorganic metal compounds, the properties of both the organic moieties of these compounds and their inorganic components would need to be addressed. Frameworks and associated guidance documents for assessing properties of organic compounds have already been developed (US-EPA, TGD guidance) and do not need to be discussed further here. Therefore, the MERAG document addresses only those assessment issues associated with inorganic metal compounds. For the purpose of this document, the term 'metals' generally refers to metals and metalloids.

It should be noted that the evaluation of toxic effects through exposure via air (important for Hg and Se) and groundwater are also not addressed in the current guidance document. New emerging concepts such as metallo-region approach and the application of the developed guidance for alloys have been indicated when appropriate but have not been extensively covered.

Finally, some elements of the risk assessment process that are not unique to metals have been covered because some metals are data-rich substances, which indirectly influences how a risk assessment can be conducted (e.g., probabilistic techniques) for these type of substances.

## 2.2 Organization

MERAG follows a 'building blocks' approach (see Figure 1) to ensure that its methodology is useful for different jurisdictions and various applications, such as chemicals management assessments and setting environmental quality objectives.

The following key building blocks can be distinguished:

### General concepts and principles

Environmental risk assessments of metals are characterized by a set of unique properties that require additional specific considerations – e.g., they are naturally occurring substances, some of them are essential, etc. The purpose of this building block is to give an overview of the main concepts and scientific principles underlying or supporting the metal-specific methodologies that have been developed in the guidance document. Concepts such as natural background, essentiality and bioavailability are discussed in depth.

### Classification

This building block is related to issues of the classification or prioritization of metals. Guidance is given on how to interpret transformation or dissolution data and which effects data are suited for incorporation in classification or prioritization schemes.

### Effects assessment

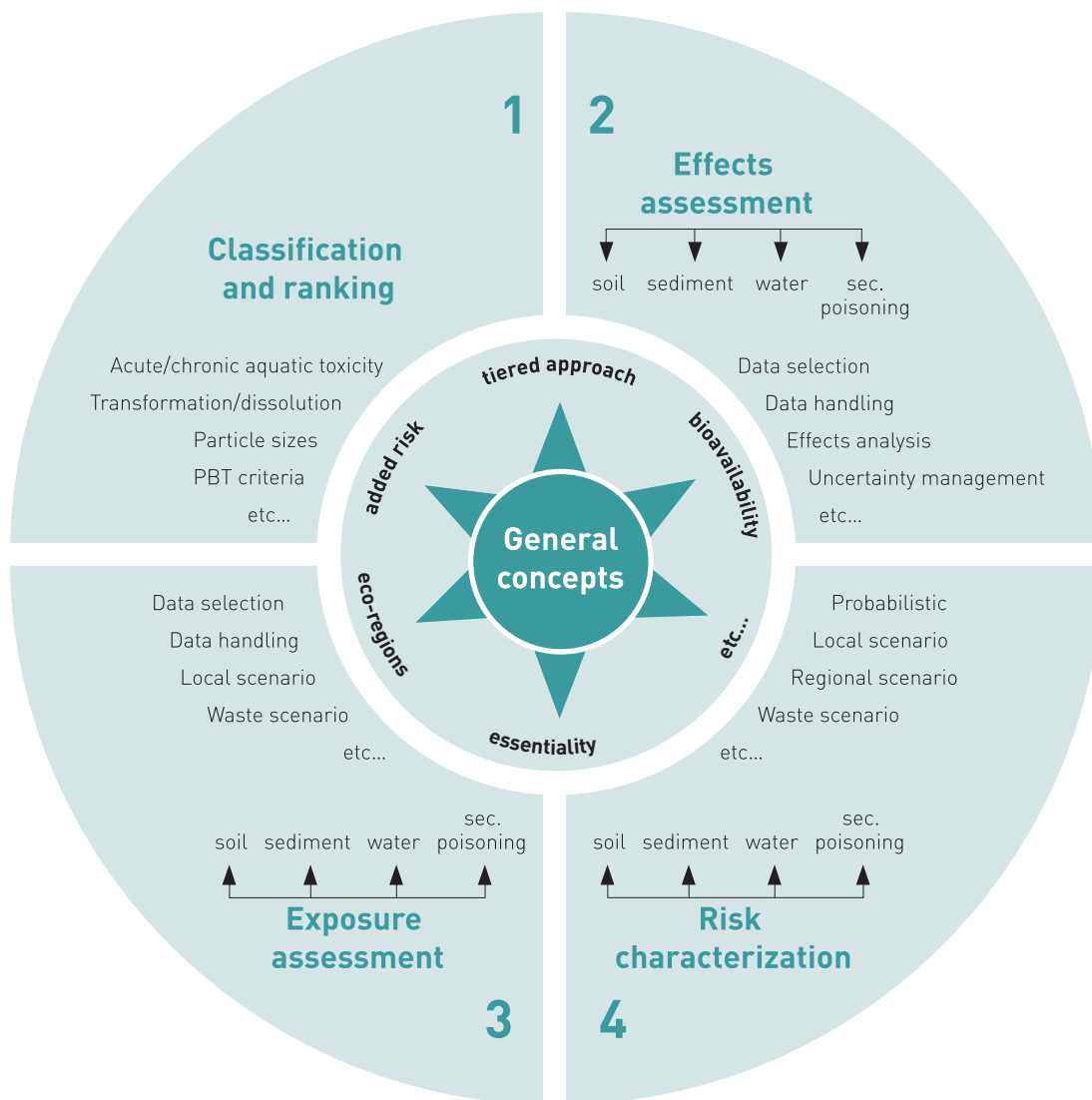
This building block is mainly concerned with setting up ecologically relevant threshold values for protecting a particular environmental compartment. In this regard, depending on the data richness, specific recommendations are provided on how to select or handle toxicity data and on how to derive or validate environmental threshold values such as the Predicted No Effect Concentration (PNEC).

### Exposure assessment

Basic recommendations on how to perform continental, regional and site-specific exposure assessments are covered by this building block. Emphasis is on the proper data selection and handling in order to derive reliable and relevant environmental concentrations from both modelled and measured data. In addition, guidance has been developed on the need for and use of specific exposure scenarios (e.g., waste scenario).



**Figure 1: Overview of key building blocks**



### Risk characterization

Finally, attention is given to how environmental risks from metals can be assessed in the most appropriate way, depending on the availability of data. In this regard the way bioavailability corrections could be incorporated in the process has been developed. The usefulness and applicability of newly developed approaches such as probabilistic risk assessments for data-rich substances are also highlighted, together with methodologies quantifying the uncertainty embedded in any risk assessment process.

These building blocks have been tackled in a series of fact sheets (presented as pdf files on the included CD-ROM) which are the core guidance elements providing the reader with state-of-the-art techniques and tools for assessing metals.

The proposed tiered approaches in the fact sheets are focused on data collection, testing strategies and risk characterization and are aimed at facilitating the risk assessment process and the setting of ecologically relevant environmental quality criteria. This approach enables the reader

to find the appropriate guidance for the data richness of the compound of interest.

In addition to the detailed prescriptive guidance given in the fact sheets, the subsequent chapters provide the reader with the main concepts and scientific principles underlying or supporting the metal-specific methodologies referred to in the different fact sheets. These general chapters address issues such as natural background, essentiality, acclimatization or adaptation and also those factors that govern metal bioavailability in the different environmental compartments (water, sediment and soil). Limitations of current methods, any new methodology currently under development and suggestions of where improvements in the process could be made in the future have been highlighted whenever possible.



METALS ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

# METALS ARE AN INTRINSIC PART OF THE ENVIRONMENT

# 03



# 03. Metals are an intrinsic part of the environment

## 3.1 Introduction

Metals – like some organic compounds (e.g., PAHs, dioxins and certain pesticides such as pyrethrins) – are naturally occurring substances and as such they can always be found at natural ('background') concentrations in the different environmental compartments. The natural background of a metal in the environment can be defined as the metal fraction that originates from natural geological, biogeochemical and other processes. For soil, the natural background is determined by the metal content of the parent soil material, the removal by biological (uptake by plants) and physicochemical (runoff, leaching to groundwater) processes, the input by organic material (remains of plants) and air deposition. For water and sediment, the background concentration is determined by the metal content, the geochemical properties of the area through with the waterbody flows, introduction of natural organic material (leaves), erosion from natural (uncontaminated) river banks and atmospheric deposition from natural origin. Due to local or regional differences in geochemistry, metal background concentrations can be quite different at different sites and scales. A local or regional environmental compartment (water, soil and sediment) is thus not characterized by one single background concentration but instead is represented by a background concentration range for each metal. This variation in natural background levels in space and time for any specific metal may have a significant impact on both exposure- and effect-related issues such as local and regional exposure assessments, essentiality issues, acclimatization/adaptation etc., and should be taken fully into account in the risk assessment process.

These related concepts – the direct and indirect consequences of the presence of a natural background – are highlighted in the next sections.

## 3.2 Essentiality

An element is considered essential when it is present in living matter, it is able to interact with living systems and a deficiency results in a reduction of a biological function that is preventable or reversible by physiological amounts of the element (Mertz, 1974). Several metals (e.g., sodium, potassium, magnesium, calcium) occur in large concentrations in organisms. A second set of metals, called trace metals, occur at much lower concentrations (usually <0.01 %) in organisms. During evolution, some of these metals have become essential for normal metabolic functioning. Essential elements (EE) are required by all organisms to grow and develop well. Two separate functions of EEs can be distinguished: (1) metals involved in electron transfer processes (e.g., copper, iron, molybdenum) and (2) direct participation of the metal in reaction mechanisms (e.g., cobalt, zinc). Several metals are essential for various biological functions, such as enzymatic and metabolic reactions. Some metals, such as Fe, Mn, Zn, Cu and Mo, are essential for almost all living organisms, while the essentiality of other metals, such as Ni, Cr and Se, has been established for a limited number of species. For other metals, no biological, nutritional or biochemical function has (yet) been identified and these are termed non-essential (Janssen and Muysen, 2001; Bossuyt, 2004). Sometimes a third class is defined as metals being beneficial but not essential (e.g., Co and Se in plants; V in animals) (EPA, 2004).

According to Liebig's law, a concentration range within which the requirements of the organism for a given EE are fulfilled can be observed for each organism and each EE (a so-called window of essentiality) (Hopkin, 1989). Within this window, organisms can regulate their internal EE concentration without experiencing excessive stress. Homeostatic mechanisms to keep the internal EE concentrations at the required levels have been developed throughout all taxonomic groups, and these homeostatic mechanisms determine the width of the window of essentiality. The species' homeostatic capacity allows it to regulate actively its internal EE concentration to a certain extent and to maintain it at optimal levels under varying external essential element availabilities. Therefore, this window is also referred to as the Optimal Concentration Range for Essential Elements (OCEE) (Van Assche et al., 1997; Muysen, 2002). This regulation, however, has limits: when the external EE concentration



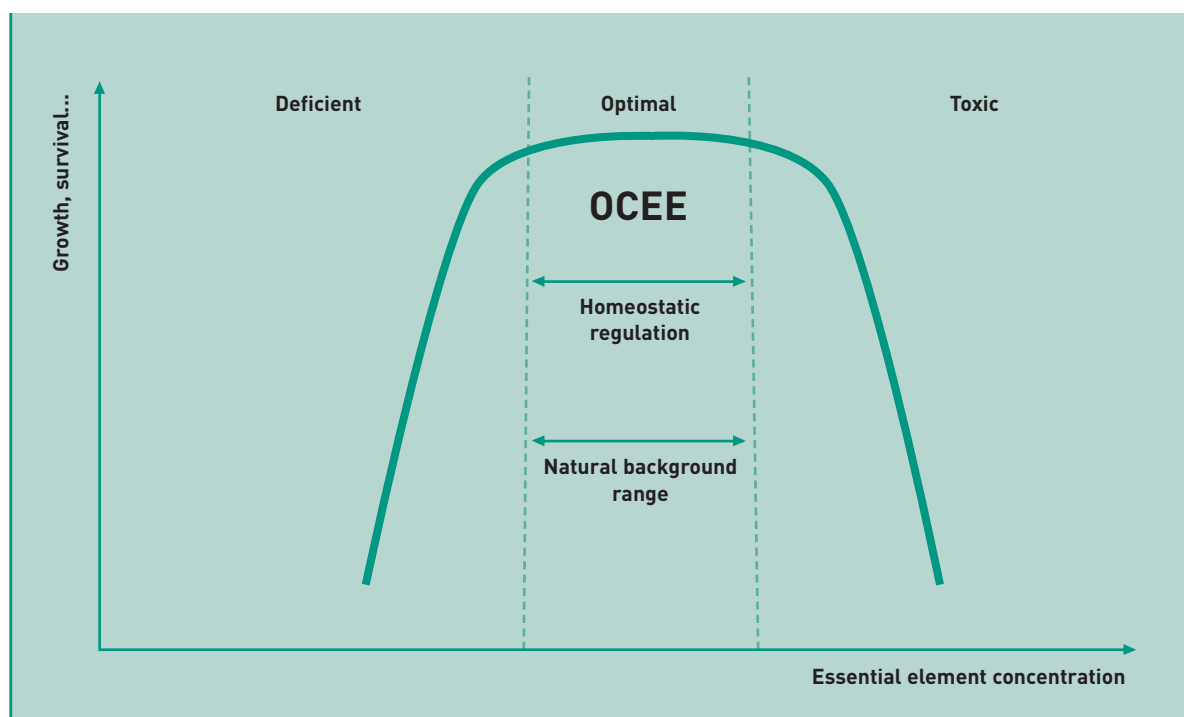
becomes too high or too low, toxicity or deficiency can occur. In general, the window of essentiality is situated in the typical range of (varying) bioavailable natural background concentrations. The OCEE is determined both by the natural concentration range of the essential element in the species' natural habitat and the species' homeostatic capacity that allows it to regulate its internal essential element levels to a certain extent (Figure 2). The mechanisms of homeostasis are described in detail in Kapustka et al. (2003).

The OCEE of a given species is related to the bioavailable EE concentration range in a certain ecosystem. Different species within a common ecosystem may have different OCEE ranges. Further, OCEEs can vary for a given species, depending on the habitat or region, due to different background concentrations of EE in different habitats or regions. This is explained in more in detail in section 3.5.

For each organism, Predicted No Effect Concentrations (PNECs) or other environmental

quality standards should be compared with the OCEE, meaning that toxicity thresholds for EEs have to be at the upper boundary of the OCEE (i.e., where toxicity occurs). Too low values will give rise to quality standards, causing deficiency. In any case, PNECs and quality standards should be validated against the lower boundary of the OCEE as minimum threshold. Therefore, OCEE curves for the different species tested are essential for sound quality criteria. For plants and wildlife, OCEE curves can for instance be derived from the available deficiency-toxicity literature, from dietary requirements of EEs for livestock or minimum requirements for plants (e.g., Epstein 1965, 1972). Ideally, a thorough comparison between toxicity thresholds/quality standards and requirements of EEs has to be made for each species, and no extrapolations between species should be performed. Additional testing will be required for a number of species and elements for accurate determination of dose-response curves with deficiency and toxicity boundaries.

**Figure 2: The Optimal Concentration range for Essential Elements (OCEE) for a species in a given habitat-type (adapted from Van Assche et al., 1997)**

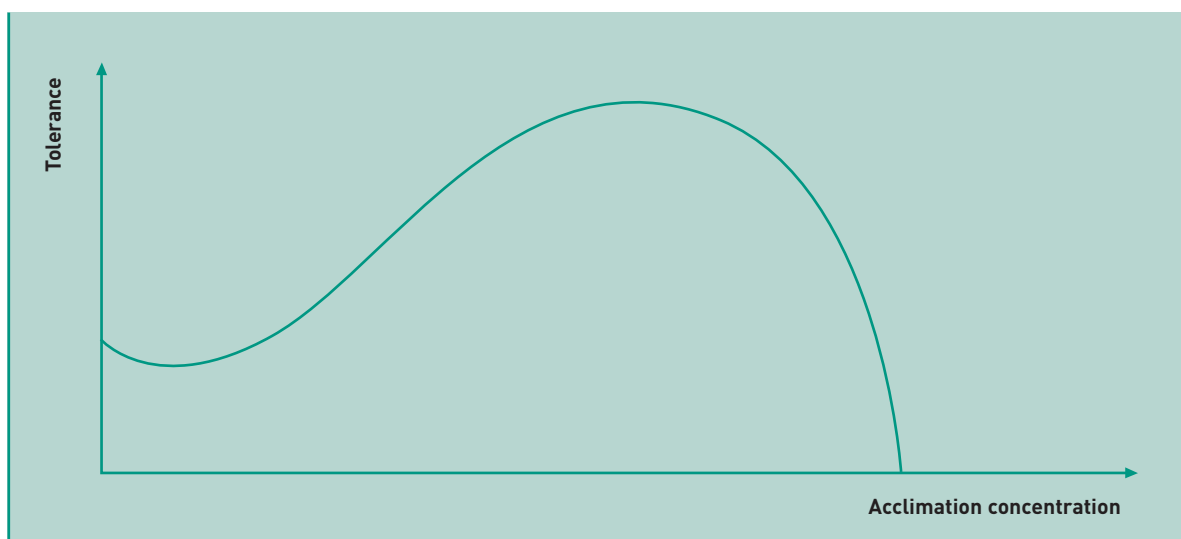


### 3.3 Adaptation/acclimation

Due to the ubiquitous presence of metals in the natural environment, organisms have become adapted during the course of evolution to the natural background concentration and have developed the capacity to cope with natural variations (e.g., seasonal changes or fluctuations in river flow rates, different metal background concentrations). Specific detoxification mechanisms such as metallothioneins, granules and others have therefore been developed in organisms. Furthermore, species have the ability to adapt or acclimate to changing metal (background) concentrations, to a certain extent. For this reason, the natural background level reflects the theoretical lower limit of the PNEC a threshold concentration, which does not present a risk to the survival of the species. This theory is applicable for all metals and is even more crucial for essential elements because the sensitivity of organisms to EEs is determined to a large extent by the bioavailable EE level that the organism experienced before testing.

Organisms are subject to deficiency or toxicity stress when external (bioavailable) EE concentrations fall outside the window of essentiality. For non-EE, only excess environmental concentrations will cause adverse effects. For both metal groups, there is a clear concentration window within which the internal (cytosolic) metal concentration of the organism is regulated without resulting in detrimental effects (although, at least theoretically, there is some metabolic cost to this). However, plants and animals tend to acclimate or adapt to low or high metal levels (both EE and non-EE), becoming more or less sensitive to the metal concerned. Acclimation involves a shift in stress tolerance of an *organism* by adjusting physiological or biochemical mechanisms; adaptation means a shift in stress tolerance of *populations* through gene selection (Benjamin and Klaine, 1995). In other words, adaptation is passed to subsequent generations, while for acclimation the offspring must also be pre-exposed to acquire it. Adaptation is thus a genetic process, beyond the lifespan of the individual, and may occur without appreciable

**Figure 3: General response model: effect of metal acclimation on subsequent tolerance to acute metal exposure (adapted from Chapman, 1985)**







metabolic cost, though perhaps with a cost to the population in terms of loss of individuals or genetic diversity (Chapman et al., 1998). Acclimation is a physiological or structural mechanism of gaining increased tolerance within the lifespan of the individual, and it may have appreciable metabolic cost. Generally, acclimation to metals is better documented than the occurrence of adaptation. The most important reason for this is that acclimation can be easily induced in the laboratory by pre-exposure to the metal over only several hours or a few weeks. In most cases where acclimation is reported, it was induced by exposure to high metal concentrations (comparable to those found at contaminated sites). New approaches, such as micro-arrays, may provide more insight into this genetic adaptation. In general, the change in tolerance is dependent on the acclimation concentration (Bossuyt, 2004). Acclimation or adaptation of organisms to high or low concentrations of metals leads – within certain concentration limits – to higher or lower tolerances, respectively (Muysen, 2002). This is presented in Figure 3. Sometimes, the response pattern consists primarily of a zone of increased tolerance bounded on either side by zones of decreased tolerance (e.g., Chapman, 1985). The existence of a lower boundary has been shown in a study of copper-acclimated rainbow trout (Dixon and Sprague, 1981) and probably results from metal uptake insufficient to trigger the induction of detoxification mechanisms (Bossuyt, 2004).

The concepts of acclimation and adaptation are not only valid for field populations, they also apply to laboratory populations. As a consequence, laboratory populations could shift to either higher or lower tolerance towards metals. This is especially the case for animals cultured or tested in artificial media, since these media contain no or very little (essential) micronutrients. The magnitude of increased tolerance may be metal-specific. Compared with other toxicants (e.g., insecticides), acclimation to metals is of much smaller magnitude (Chapman, 1983).

Acclimation and adaptation are based on the development of new or the induction, amelioration or expansion of existing detoxification mechanisms. Different mechanisms have been described: metal exclusion (with or without the release of extracellular metabolites; Foster, 1977; McKnight and Morel, 1979; Fisher and Fabris, 1982); active transport (perhaps restricted to certain essential

trace metals; Rainbow and Dallinger, 1993, cited in Bossuyt, p. 19); extracellular sequestration; intracellular sequestration (through protein binding, by means of phytochelatins or metallothioneins; Grill et al., 1985; Gekeler et al., 1988; Roesijadi, 1992); enzymatic detoxification and bypassing (alternate pathways) sensitive components and sequestering of metals in cysts, granules or vesicles within or outside the cells (Silverberg et al., 1976; Brown, 1977; George and Pirie, 1979; Lowe and Moore, 1979; Mason et al., 1984; Bardeggia and Alikhan, 1991). In addition to function in the regulation of essential metals, metallothioneins (in animals and bacteria) and phytochelatins (in plants, algae and some fungi) are also involved in the detoxification of essential and non-essential metals, due to their high affinity for metals (Roesijadi, 1992; Ahner and Morel, 1995).

Adaptation to metal exposure may improve fitness of the individual in contaminated habitats, but several potentially deleterious consequences are identified, which determine population persistence at contaminated sites. Maintenance of tolerance mechanisms may be physiologically or energetically costly, so that allocation of nutrients or energy to other functions is reduced ('cost of tolerance') (Posthuma and Van Straalen, 1993). Other documented negative consequences of acclimation/adaptation include a decrease of genetic variation and the possibility of bioconcentrated metals being passed on to non-resistant consumers (Posthuma and Van Straalen, 1993; LeBlanc, 1982). The reduced genetic diversity may negatively influence the population's resistance to other stressors.

### 3.4 Toxicity of EEs within the framework of environmental quality criteria setting

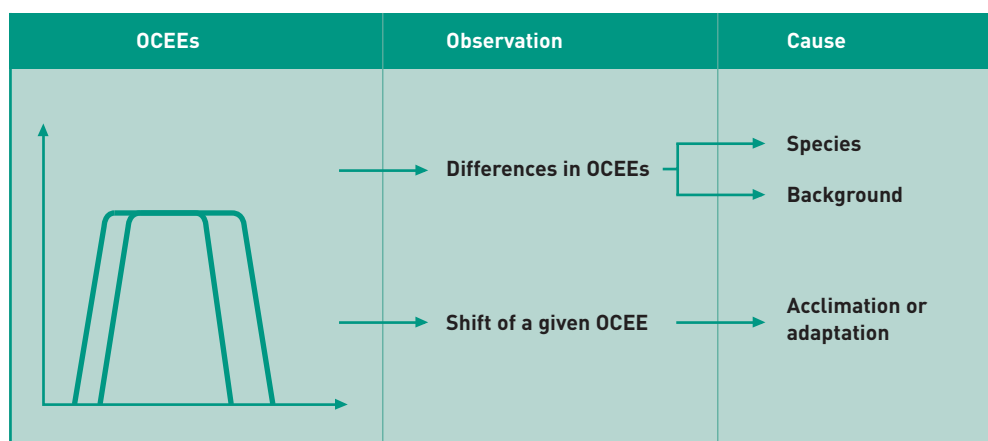
It is essential to consider the fact that the sensitivity of organisms to EEs in toxicity tests is determined to a large extent by the EE concentrations before the testing. Moreover, organisms cultured in media with low EE concentrations may exhibit an overall decreased fitness (e.g., Eisler, 1993; Van Tilborg and Van Assche, 1995; Dixon and Sprague, 1981). Furthermore, organisms cultured at these low metal concentrations acclimate to these conditions and become more sensitive to stress, including exposure to metals. Conversely, organisms cultured in media with elevated metal concentrations (e.g., natural waters or contaminated waters) may become less sensitive (Muysen, 2002). This means that results from tests with EEs are only applicable to environments where similar (bioavailable) background concentrations are available. The use of test results in different environments is not relevant or must take into account background concentrations and acclimation or adaptation. Therefore, culture and test conditions (constitution of media) must be described in detail. Culture media for test organisms should ideally reflect the natural environment, and addition to culture media of compounds present in a natural (unpolluted) environment (e.g., essential and non-essential metals) should be considered. Consequently, only data sets with clearly defined, relevant conditions should be withheld for derivation of, e.g., PNEC or

HC<sub>5</sub>. As guidance, the minimal EE concentration not causing deficiency for the test species used (lower boundary of OCEE), could be added to breeding and test media. In case of multi-species tests (microcosm, mesocosm), the lower boundary of the No Risk Area (NRA, see later discussion) of the species used, could be added to culture and test media.

However, it is difficult to conduct pre-acclimation for soil microbial studies because *in situ* populations of microorganisms from previously collected soils are used. This calls into question the applicability of results from microbial function studies (USEPA, 2003), and until these issues are resolved, the use of results from such studies for risk assessment is questionable.

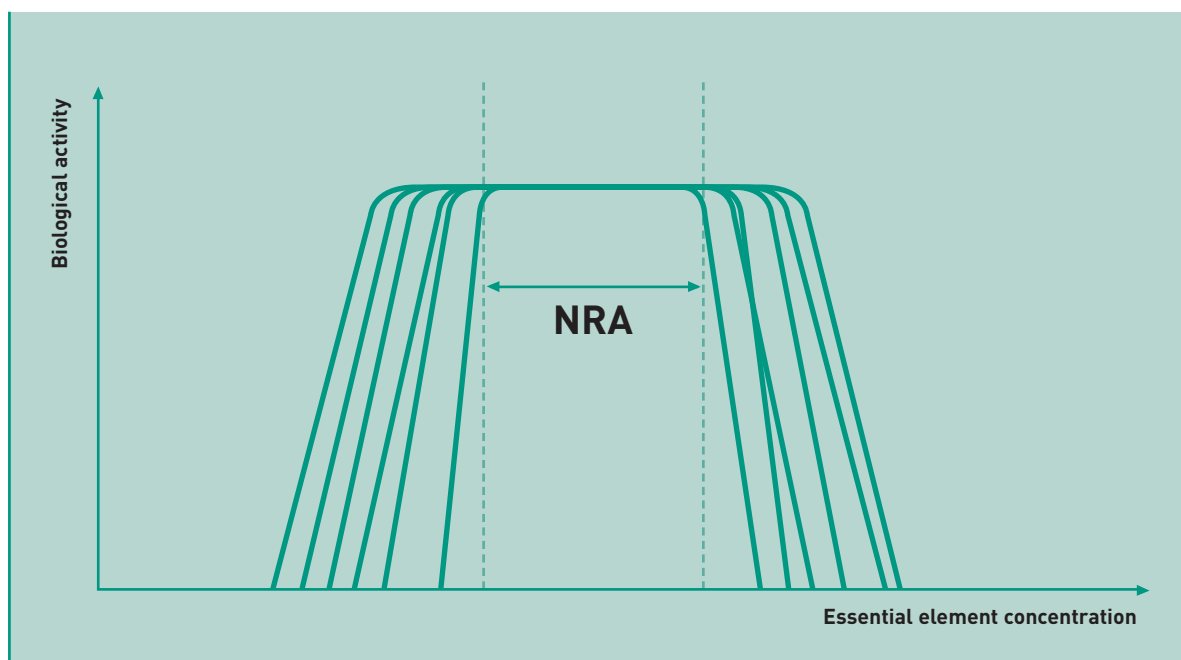
If necessary or helpful, grouping of test results according to the background EE concentrations in the culture and test media should be performed, and laboratory results should only be used in environments with similar background concentration ranges. In this respect, the metalloregion concept could possibly be used (accounting for background concentrations and bioavailability factors, see section 3.5). The same applies for the species tested. It is strongly recommended that only results of experiments with species relevant to the (metallo)region of concern be used in toxicity testing and quality criteria setting. Differences and shifts in OCEEs are given in Figure 4.

Figure 4: Causes of differences and shifts in OCEEs





**Figure 5:** Hypothetical presentation of the OCEE curves of all organisms in a given environment. The inner envelope of these curves represents a No Risk Area in which all organisms are protected from both deficiency and toxicity effects (Janssen and Muysen, 2001)



Current methods for PNEC predictions include the use of safety factors and statistical extrapolation techniques (species sensitivity distributions). Applying these techniques to EEs leads to a number of problems. The safety factor approach often leads to PNECs well below the EE's natural concentration range and would therefore be situated at concentrations that are deficient for (some) organisms in a given ecosystem. Statistical extrapolation techniques, on the other hand, use statistical distributions that do not consider possible adverse effects due to deficiency (Muysen, 2002), and resulting PNECs may be at the lower end of (or beyond) the homeostasis range of some organisms. Therefore, the No Risk Area was proposed by Van Assche et al. (1997) as a basis for PNEC determination for EEs and for dealing with different OCEEs for different species. The NRA is determined by the inner envelope of the overlapping OCEE curves of a group of species belonging to a given habitat-type/metalluregion (Figure 5).

Within the NRA, none of the species is subjected to either deficiency or toxicity stress. The NRA's upper boundary is determined by the biological species with the lowest toxicity value, with the deficiency boundary determined by the species

with the highest deficiency values (Janssen and Muysen, 2001). As an alternative (if too few data are available to determine the NRA), the 90th or 95th percentile of the range of natural background concentrations in the region of interest could be determined. This value would then act as a reasonable lower limit for the PNEC.

### 3.5 The metalluregion concept

The metalluregion approach arises from the fact that different ecoregions can be identified based upon soil type, climatic factors, latitude and elevation (Waeterschoot et al., 2003; Bailey, 1998). Within ecoregions, sub-ecoregions (also called metalluregions) can be differentiated based upon the natural background concentration of the metal under consideration and the presence of well-defined abiotic factors that influence metal bioavailability. However, metalluregions are not likely to be completely contiguous with ecoregions and must be derived using similar methods of aggregating spatially explicit environmental variables. Apart from metal background concentrations, different controlling factors must receive consideration (e.g., Ca and Mg concentrations, pH, buffering capacity for water;

CEC, organic matter; pH for soils) (Fairbrother and McLaughlin, 2002). For zinc, for example, the lowest concentrations are found in the open ocean (0.001–0.06 µg/l). In uncontaminated freshwater environments, zinc concentrations range from 0.09–0.3 µg/l (large lakes, e.g., Great Lakes in US; Nriagu et al., 1996) to over 5–40 µg/l (alluvial lowland rivers, e.g., European lowland; Zuurdeeg, 1992) and over 200 µg/l in streams enriched in EE and flowing through mineralization areas (Whitton et al., 1982). Using available GIS technology, metalloregions can be developed relatively easily as further refinements of existing ecoregions.

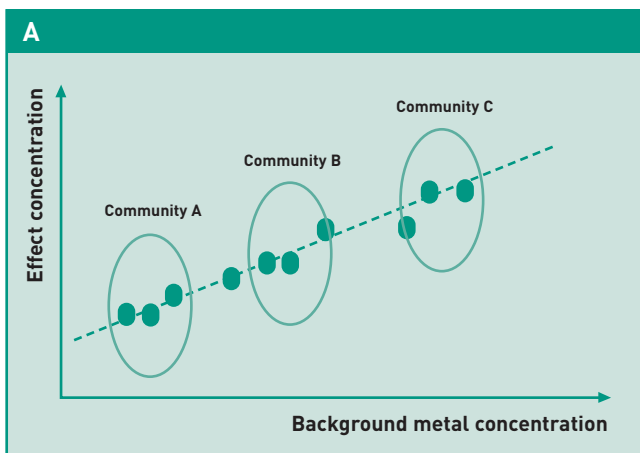
According to Fairbrother and McLaughlin (2002), the metalloregion concept is most closely aligned with a spatially nested hierarchical classification (Klijn, 1994; Fairbrother and McLaughlin, 2002), with differing thematic overlays and a multivariate classification analysis to minimize variability within groups and to maximize variability amongst groups. The data need to be appropriately qualified to differentiate natural background levels from those that have been anthropogenically elevated prior to use in delineating natural metalloregions. Alternatively, metalloregions could be defined on the basis of current ambient conditions, excluding excessively polluted locations. This applies to the soil, water and sediment compartment.

The method of choice for metalloregion delineation has a marked impact on metalloregion boundaries. Many ecosystems have been disturbed greatly from the natural state by human activities. For example, large areas of infertile acidic soils have been turned into agriculturally productive systems through inputs of lime and fertilizers. Despite similar climates and 'background' soil characteristics between the natural and modified land uses, these systems would be identified as separate metalloregions based on ambient (i.e., current) soil conditions (Fairbrother and McLaughlin, 2002). In other cases, the choice for current ambient conditions or 'natural background conditions' is less obvious. Furthermore, the 'natural background' can very difficult to determine. The metalloregion concept thus provides a conceptual framework to account for the broad regional parameters affecting metal availability.

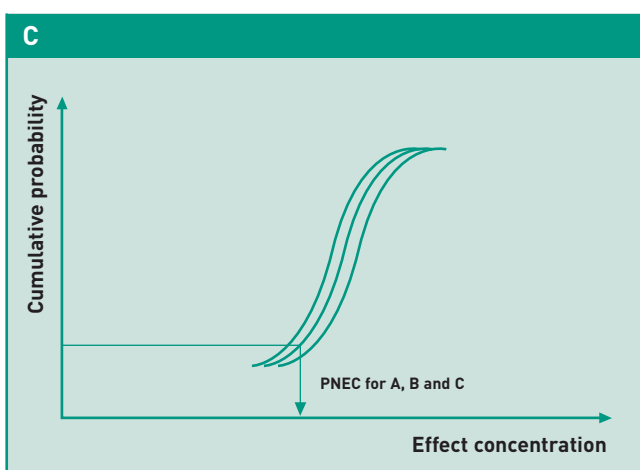
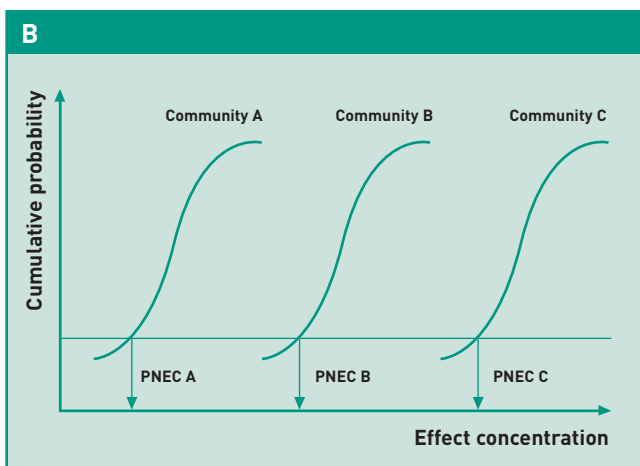
This concept also recognizes that (bioavailable) background concentrations of a metal in a given ecoregion can differ from one ecosystem to the next, resulting in different sensitivities to the toxic effects of metals due to acclimation or adaptation. In this way, effect datasets can be divided into different metalloregion groups. Typical metalloregions for the metal under consideration based upon clearly distinguished ranges of natural background concentrations must be defined, and PNECs should be derived for each of these metalloregions (Waeterschoot et al., 2003). A PNEC derived for a metal-deprived area will not be suitable for a metal-rich area. In other words, different screening criteria are needed for each region when assessing risk from metals, and region-specific PNECs should be derived, based on testing conducted with regionally relevant organisms that are acclimated or adapted to the background levels of metals within each region. Ideally, appropriate organisms can then be cultured and tested in water or soil of the (metallo)region investigated (Fairbrother and McLaughlin 2002). The metalloregion concept thus accounts for regional parameters affecting metal availability in soil, water and sediment, as well as for the differences in organisms to respond to metal addition.

Figure 6 illustrates this for a single species living at various background concentrations: as the background concentration of the metal increases (within natural limits), the sensitivity (to the metal) of the acclimated or adapted organism decreases. Expanding this hypothesis to all organisms of an aquatic community living in a specific metal background concentration range, two hypothetical cases may be discerned (Janssen et al., 2000).

The shift in the communities' sensitivity due to the acclimation or adaptation process is larger than the span of the species' sensitivity distribution, resulting in a distinctive sensitivity of each community – i.e., little or no overlap of the Species Sensitivity Distribution (SSD) (Figure 6B). In this case, different water quality criteria or PNECs for different metalloregions should be derived. If the species sensitivity distributions largely overlap – i.e., acclimation-induced sensitivity shifts do not or only slightly affect the tolerance of most species in the community – the proposed hypothesis should be rejected and may not be useful in regulatory context (Figure 6C).



**Figure 6:** Hypothetical relationship between the sensitivity of a single species (A) or different communities (B and C) and the background concentration of an essential metal (from Janssen et al., 2000)



The metalloregion concept may be used effectively in different ways. First, a classification may be developed that is based on water or soil types, thus including both the metal background concentrations and the factors influencing bioavailability. This classification should contain models describing the effect of acclimation and bioavailability changes, allowing 'translation' of standard toxicity results into data that are related to a certain metalloregion. Secondly, toxicity data should be derived from tests, also taking the following into account:

- If standard test organisms are used, they should be cultured at bioavailable metal background concentrations that are representative of the environmental system under consideration.
- The dilution and control media should contain the same representative background concentration.
- The dilution medium can be a well-described natural medium representative of the system.
- Test organisms may be collected in a representative natural environment, cultured on the natural medium.
- Ecotoxicity tests can be conducted in well-described natural waters.
- To be environmentally realistic, organisms should be fed with contaminated food (i.e., food that was exposed to the same metal concentration).
- Tests should be conducted at a constant temperature, and protocols should acknowledge that temperature affects bioaccumulation and effects.



METALS ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

# SPECIATION, MOBILITY AND BIOAVAILABILITY OF METALS IN THE ENVIRONMENT

# 04



# 04. Speciation, Mobility and Bioavailability of Metals in the Environment

There is extensive evidence that neither total nor dissolved aqueous metal concentrations are good predictors of metal bioavailability and toxicity (Campbell, 1995; Bergman et al., 1997; Janssen et al., 2000), and the importance of explicitly considering bioavailability in the risk assessment of metals in general and the development of water and sediment quality criteria in particular has been demonstrated scientifically (Di Toro et al., 1991; Ankley et al., 1996; Allen and Hansen, 1996; Janssen et al., 2000). Recently, these concepts and the scientific methods to assess bioavailability of metals are increasingly recognized by regulatory authorities (Renner, 1997; Bergman et al., 1997).

It is now clear that the dissolved free ionic metal species is far more bioavailable than most complexed metal species. Templeton et al. (2000) defined a metal species as a “specific form of an element defined as to isotopic composition, electronic or oxidation state, complex or molecular structure”. It is also well established that many geochemical factors influence the metal speciation in water, sediment and soil. Metal speciation is “the distribution of an element amongst defined chemical species in a system” (Templeton et al., 2000) and is the result of one or more chemical analyses (speciation analysis) or chemical modelling. Paradigms that explain the relationships between these geochemical factors and metal bioavailability and toxicity are either well developed (the case for freshwater and sediment compartments) or are under development (the case for soil and marine compartment). Metal speciation programs such as the Windermere Humic Aqueous Model (WHAM) and more complex models such as the Biotic Ligand Model (BLM) integrating biotic and abiotic factors have proved to be useful for surface waters in this aspect. For soils, bioavailability approaches are presently more empirically based. For sediments, the Simultaneously Extracted Metal–Acid Volatile Sulfides (SEM-AVS) concept has proved useful for metals that are susceptible for binding with sulfides. For those metals where SEM-AVS is not applicable, the use of partitioning to Fe-Mn (oxy)hydroxides or species transformation (reduced forms under anoxic conditions) as an alternative approach for non-sulfide binders gained more attention.

Incorporation of bioavailability should be done on both the effects and the exposure side when transformation to a dissolved fraction or metal speciation models is used. However, when toxicity-based bioavailability models are used (e.g., the

Biotic Ligand Model) the correction for bioavailability should preferentially be applied at the effect side: here, the bioavailable fraction not only depends on the environmental conditions, it is also organism-dependent. Indeed the effect of pH or increased cation concentrations on metal toxicity can be different for various organisms.

In the next sections the underlying concepts, scientific evidence and possible merits of introducing these bioavailability concepts into the risk assessment process or environmental quality criteria setting are discussed for water, sediments and soil.

## 4.1 Speciation/bioavailability of metals/metal compounds in the water compartment

### 4.1.1 The Free Ion Activity Model

It has long been recognized that the formation of organic and inorganic metal complexes and metal sorption to particulate material reduces metal bioavailability and toxicity in the water compartment (Pagenkopf et al., 1974; Sunda and Guillard, 1976; Sunda and Hansen, 1979; Pagenkopf, 1983 in Di Toro et al., 2001). As a result, the relationship of metal toxicity to total or dissolved concentrations can be highly variable, depending on ambient water chemistry (Di Toro et al., 2001). Initially the free ion activity was considered a better measure for metal toxicity, resulting in the formulation of the Free Ion Activity Model (FIAM). This model describes how variations in the effect levels of metals can be explained on the basis of metal speciation and metal interactions with the organisms (Morel, 1983; Paquin et al., 2002). The concepts of the FIAM in aquatic toxicology have been comprehensively reviewed by Morel (1983) and Campbell (1985) and were re-evaluated more recently by Brown and Markich (2000). The early conceptual description of the FIAM incorporated both the binding of the free metal ion  $Me^{2+}$ , generally considered to be the most toxic metal species (Starodub et al., 1987), and other metal complexes to cellular sites at the site of toxic action, and it also took competitive effects into account. A specific description of how the FIAM would be applied, however, was not provided.

Different parameters affect metal speciation or bioavailability in surface waters: pH and redox potential (Eh), major cation concentrations, alkalinity, ionic strength and complexing ligands (organic, inorganic ligands). The effects of the most important parameters are briefly described here.





### pH and redox potential (Eh)

The pH and Eh of water control the solubility and concentration of the major metal species (e.g., Campbell and Stokes, 1985; Cusimano et al., 1986; Evans et al., 1988; Farag et al., 1993; Kimball and Wetherbee, 1989; Pagenkopf, 1983; Schubauer et al., 1993). The fraction of a specific metal species (e.g.,  $Me^{n+}$ ) at a specific pH level, however, is metal-dependent.

An increase of the acidity results in an increase of the free metal ion concentration in solution, due to competition between bound metal ions and free protons for the negatively charged exchange sites in organic and inorganic ligands. Acidification can affect not only metal complexation equilibria in solution, but also metal interactions at organic or biological surfaces – i.e., protons can exert a competitive benefit, resulting in a reduced metal toxicity (Campbell and Stokes, 1985; Cusimano and Brakke, 1986). The concentration of the various (in)organic cations and anions determine which metal-complex species will occur in water.

### Hardness and major ion concentrations

Hardness, mainly determined by calcium ( $Ca^{2+}$ ) and magnesium ( $Mg^{2+}$ ) in most natural waters, affects ecotoxicity and bioavailability by competing with metals for binding on dissolved organic matter, anions and biological membranes (e.g., gills) (Benson and Birge, 1985; Hodson et al., 1978; Lauren and McDonald, 1986; Pagenkopf, 1983). Increased concentration of hardness ions can therefore interfere with an organisms' ability to take up potentially toxic trace metals.

The metals most affected by hardness include cadmium, chromium, cobalt, copper, manganese, nickel and zinc, but sufficient data have not yet been obtained for some to describe hardness-toxicity equations (e.g., Co, Mn) (e.g., Bradley and Sprague, 1985; Chapman et al., 1980; Pagenkopf, 1983; Stubblefield et al., 1991; Diamond et al., 1992).

Because variation in hardness in natural waters is often correlated with variation in pH, alkalinity and ionic strength, the observed effects on toxicity should not be confused with an effect of water hardness *per se*. For a thorough understanding of metal bioavailability, it may be necessary to consider the separate effects of the bioavailability mitigating processes rather than the combined effects (Erickson et al., 1994).

Other cations like sodium, iron and aluminium may also compete with some metals for binding to specific sites. For instance, increased sodium levels will reduce copper binding (and uptake) to biological membranes, whereas iron and aluminium form very strong complexes with organic matter due to their higher valence (III) (Tipping, 1994; Gamble and Schnitzer, 1973) and thus prevent other metals like copper from complexing with dissolved organic matter (DOM) (De Schampelaere and Janssen, 2004).

### Alkalinity

Alkalinity affects bioavailability in a manner analogous to hardness, but it involves the carbonate anions instead of the  $Ca^{2+}$  and  $Mg^{2+}$  cations: increased alkalinity will reduce metal bioavailability through the formation of metal-carbonate complexes. For metals like copper and lead, it has been found that alkalinity may have a greater influence than hardness on the bioavailability (Andrew, 1976; Nelson et al., 1986; Shaw and Brown, 1974; Davies, 1976).

### Ionic strength

The ionic strength of surface water will also influence the bioavailability of metals and metal compounds. The ionic content of waters with a high ionic strength may compete for uptake with the ionic metal species or may influence the thermodynamic equilibria altering the ratios of metal species in solution (Stumm and Morgan, 1981). This phenomenon, however, is mainly related to marine and estuarine environments.

### Dissolved organic compounds (DOM, NOM)

Natural organic matter, often dominated by humic substances (decomposing organic matter), is present in natural waters and can dominate the speciation of many metals. Metals bind to NOM-ligands primarily at carboxylic and phenolic functional groups, but other groups like amino- and sulfidic groups may also play an important role. Description of metal interactions with natural organic matter currently remains an immense challenge due to its polydisperse, heterogeneous nature (Paquin et al., 2002): as the composition of natural organic matter can be highly variable, large differences in binding capacities among various DOM may occur. Recent speciation models that incorporate multiple binding sites and competition between cations are the Windermere Humic Aqueous Model (Tipping, 1994) and the Non-Ideal Competitive Adsorption (NICA) Model (Benedetti et al., 1995).

### Inorganic ligands

The five inorganic ligands of primary importance in natural waters are  $F^-$ ,  $Cl^-$ ,  $SO_4^{2-}$ ,  $OH^-$  and  $HCO_3^-$  (Parametrix, 1995). Other important inorganic ligands are  $CO_3^{2-}$ ,  $HPO_3^{2-}$ ,  $NH_3$  and, in anoxic waters,  $HS^-$  and  $S_2$ . The effect of these ligands on metal bioavailability is often seen as a direct consequence of dissolved ligand competition with binding sites on gills or gut surface for the free metal ion. Dissolved ligands may modify metal transport across membranes through several mechanisms, including competition with surface ligand sites, modification of lipid solubility, precipitation of complexes, and modification of biological processes (e.g., osmoregulation, respiration, excretion) (Newman and Jagoe, 1994).

A classification of environmentally relevant metals and their preferred ligands is given in Table 1. This table also contains the classification of NOM-related functional groups.

### 4.1.2 Chemical speciation models

Currently there are a number of chemical speciation models or equivalent models that provide a good characterization of the metal chemical species in a solution containing inorganic ligands and well-characterized organic ligands. As binding of metals to organic matter is often one of the most dominating processes in natural water, it is essential that such speciation models include an accurate description of NOM-reactions with trace metals.

The Windermere Humic Aqueous Model (Model V) was recently developed to simulate chemical equilibrium of waters, sediments and soils dominated by natural organic matter (Tipping, 1994). The model simulates metal-organic matter interactions as a combination of chemical and electrostatic interactions. Competitive binding of metals includes monodentate and bidentate interactions. Chemical binding can be modified by

**Table 1: Classification of environmentally relevant metals and their preferred organic binding ligands (in Smith et al., 2002)**

Group A Metals	Intermediate Metals	Group B Metals
$H^+$ , $Na^+$ , $K^+$ , $Mg^{2+}$ , $Ca^{2+}$ , $Sr^{2+}$ , $Al^{3+}$ , $Fe^{3+}$	$Mn^{2+}$ , $Fe^{2+}$ , $Zn^{2+}$ , $Co^{2+}$ , $Cd^{2+}$ , $Cu^{2+}$ , $Pb^{2+}$ , $Ni^{2+}$ , $Cr^{3+}$ , $Fe^{3+}$ , $Mn^{3+}$	$Au^+$ , $Cu^+$ , $Ag^+$ , $Cd^{2+}$ , $Hg^{2+}$ , $Pb^{2+}$ , $Sn^{2+}$ , $Au^{3+}$
$F > O > N = Cl > Br > I > S$		$S > I > Br > Cl = N > O > F$
$-OH > -OAr > -OOCR$ $CO_3^{2-} \gg NO_3^-$ $PO_4^{3-} \gg SO_4^{2-} \gg ClO_4^-$		$-S, -SR, -SM$
Mainly electrostatic		Mainly covalent



electrostatic interactions, depending on the net charge of the organic molecules and the ionic strength of the aquatic simulation. Non-specific electrostatic binding is also represented using a Donnan type diffuse layer model.

WHAM was the outcome of a series of models developed to describe natural organic matter chemistry and interactions with metals:

Model I and II: development of a framework for describing the interactions of protons – Al and Ca on one hand and humic substances on the other (Backes and Tipping, 1987)

Model III: addition of site heterogeneity (Tipping et al., 1988, 1989)

Model IV: addition of non-specific electrostatic binding (Tipping et al., 1990, 1991)

Model V: incorporation of trace metal complexation (Tipping, 1993)

Model VI: addition of tridentate metal complexes and more detailed descriptions of binding heterogeneity (Tipping, 1997).

WHAM, version 1.0, formalized the trace metal complexation approach from Model V by establishing a database of best-fit parameters for simultaneous calibration to a wide array of metals (Tipping, 1994). Recently, Model VI was described (Tipping, 1998).

A second recently developed model is the CHEMical Equilibria in Soils and Solutions (CHESS) Model (Santore and Driscoll, 1995). The CHESS Model was developed in such a way that it can work as a subroutine within other models to facilitate adding chemical equilibrium and speciation calculations.

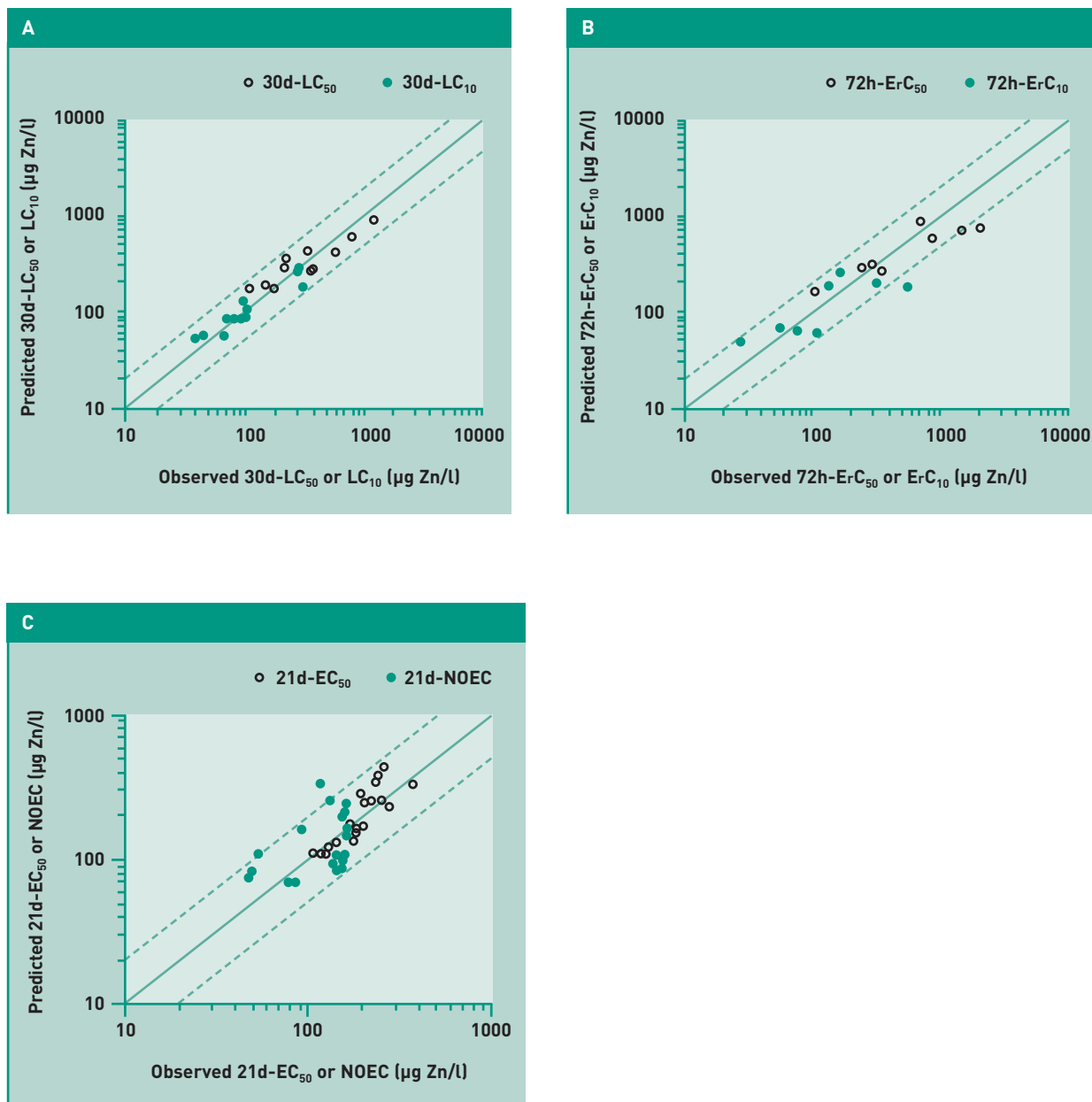
Finally, the Non-Ideal Competitive Adsorption Model (Benedetti et al., 1995) is a multi-site model that accounts for non-ideal binding of metal ions to heterogeneous organic matter. The model has been refined to include a Donnan-type formulation for non-specific binding of ions (Kinniburgh et al., 1996). The NICA-Model is somewhat comparable in structure to WHAM and has been shown to effectively simulate the binding of copper, cadmium and lead ions to dissolved organic matter (Benedetti et al., 1995; Kinniburgh et al., 1996; Temminghoff et al., 1997; de Rooij et al., 1999). As such, it may serve as an alternative to the previously described speciation models.

The capabilities of WHAM (Model V), along with extensive calibration to published data sets, make this model arguably the most comprehensive to date for simulation of metal chemistry where interactions with natural organic matter are important. The lack of a generic computation framework, on the other hand, makes it difficult to add chemical processes that are currently not represented (e.g., precipitation of insoluble metal salts). The current structure of WHAM, for instance, includes a description of the distribution of organic functional groups in the source code, making it difficult to change this distribution without altering the computer code. To alleviate such problems, the CHESS Model was recently modified to duplicate the chemical and electrostatic interactions derived for the WHAM (Santore et al., 1998; Meyer et al., 1999). The combination of these two models provides a comprehensive description of metal-organic matter speciation within a generic speciation framework. However, the accuracy of the metal speciation prediction for a specific site depends on the complexity of the environmental conditions, combined with the availability of monitoring data for (metal-specific) key parameters that determine metal speciation. Under such circumstances, a validation of the speciation model is recommended – e.g., by means of electroanalytical methods that determine one or more specific metal species or groups of metal species (anodic and cathodic stripping voltammetry, ion-selective electrodes, etc.).

#### 4.1.3 The Biotic Ligand Model

The Biotic Ligand Model concept, which has recently been applied successfully to predict metal bioavailability and acute toxicity for several metals, e.g., Cu (Santore et al., 2001; De Schamphelaere et al., 2002a,b), Ag (Paquin et al., 1999) and Zn (Heijerick et al., 2002a,b) in surface waters, is currently the most developed practically applicable technique to assess the ecotoxicity of metals on a site-specific basis. The BLM provides a mechanistic understanding of the interactions of metals with organic, inorganic and biotic ligands at the biological membrane and allows for the quantification of the bioavailability under given abiotic conditions of the water. Recently, the BLM concept has been extended for predicting chronic copper and zinc toxicity for different trophic levels (De Schamphelaere et al., 2003; De Schamphelaere and Janssen, 2004). Excellent reviews with regard to the developed BLMs have been written by Paquin et al. (2002) and Niyogi and Wood (2004).

**Figure 7:** Relationships between BLM-predicted and observed toxicity for (a) *O. mykiss* (b) *P. subcapitata* and (c) *D. magna*. Chronic Zn-BLMs were developed for the fish *Onchorhynchus mykiss*, the invertebrate *D. magna* and the green alga *Pseudokirchneriella subcapitata*. The BLM predictions significantly reduce the variability on the toxicity data used: chronic toxicity is generally predicted within factor 2 (in a few cases, factor 3) for all three trophic levels, while the variability on the toxicity data, expressed as total zinc, can be more than a factor of 100 (e.g., algae). In this case, the BLM-normalization reduces the variability by more than 2 orders of magnitude





By using the BLM, the uncertainty and variability associated with a Species Sensitivity Distribution can be reduced in a significant way. For example, with the BLM the observed variation among effect concentrations for a single test species exposed in different test media could be reduced by more than 2 orders of magnitude, as is apparent in Figure 7.

BLMs can be used to normalize the effects data of a Species Sensitivity Distribution towards similar physicochemical conditions, thus reducing the variability associated with the variation of pH, hardness, DOC, etc. among different test media. As no BLMs are available for all test species that are included in an SSD, it is assumed that existing BLMs can predict toxicity levels for species within the same taxonomical group after taking an additional calibration step into account (e.g., the BLM for the rainbow trout for all fish, the *Daphnia*-Model for all crustaceans, etc.). This so-called cross-reading implies that binding properties of metals and competing cations to the organisms should be similar within different taxonomical groups (e.g., crustaceans, fish, algae). Until now, no scientific evidence is available to confirm or reject this hypothesis. The usefulness of cross-reading has been demonstrated for copper and rotifera, for which the *D. magna* BLM was successfully calibrated (De Schamphelaere et al., 2006). It remains, however, uncertain to what extent cross-reading is also applicable for other metals and organisms (e.g., use of the fish-BLM for normalization of amphibian data).

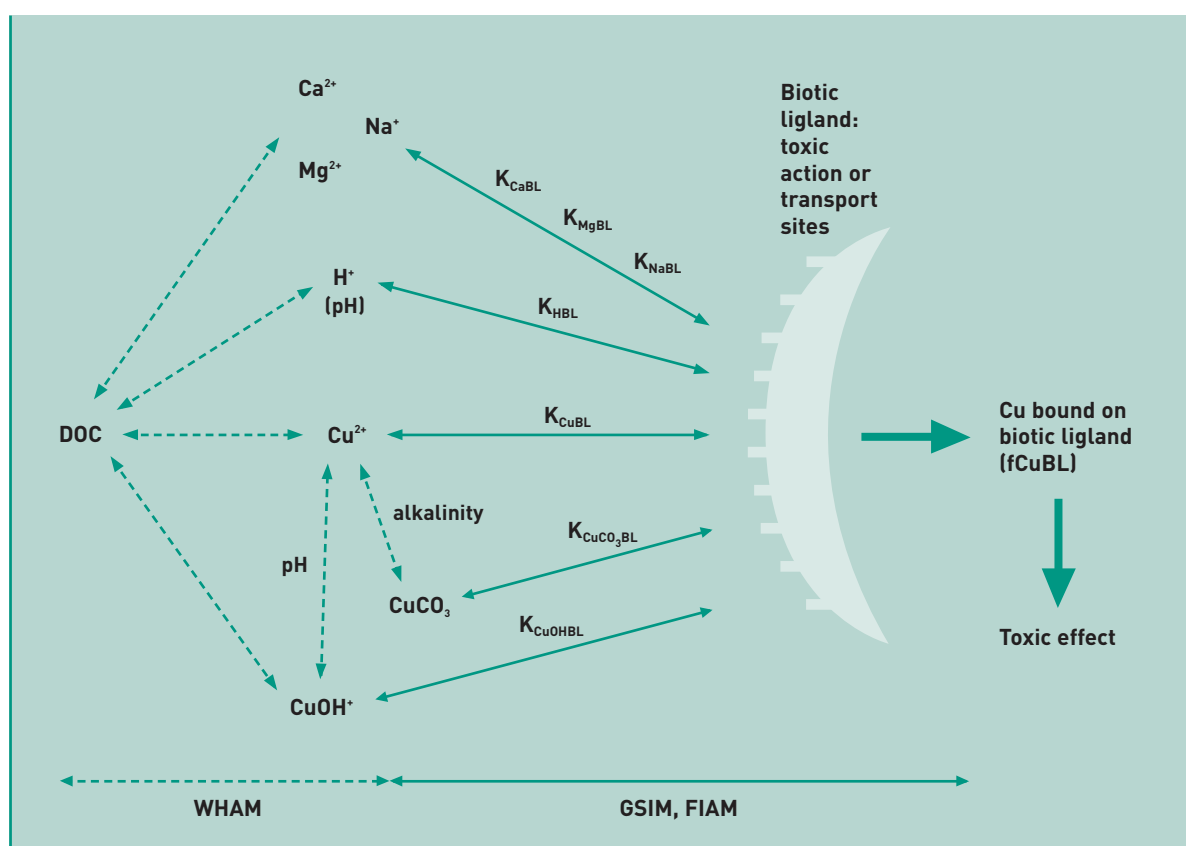
Reductions of toxicity variability have been observed in validation exercises that were undertaken with the developed BLMs for other metals (e.g., Cu, Ag; Bury et al., 2002; De Schamphelaere et al., 2002; De Schamphelaere and Janssen, 2004; McGeer et al., 2000), and the difference between observed and predicted toxicity was mostly less than a factor of 2. These findings again demonstrate the potential of the BLMs to reduce variability in reported effect concentrations when physicochemical aspects are taken into account.

The general concept of the BLM is similar to the gill site interaction model proposed by Pagenkopf (1983) and mathematically integrates the interaction of trace metals with solution phase ligands to predict its speciation and its subsequent interaction with receptor sites on the organism (Allen, 2002). The BLM thus includes two features – one biological and one chemical – that enable it to predict toxicity of dissolved metals based on the accumulation of the metal on the receptor site. From a chemical and mathematical perspective, the organisms' receptor site is treated as a ligand, the 'biotic ligand' (BL). While other models can predict metal bioaccumulation on the gill in short-term exposures (Playle, 1993a,b), the BLM is the first that includes a scheme for predicting toxicity. Central to the BLM is the notion that the chemistry of the system is at equilibrium, thus allowing the use of thermodynamic and conditional binding constants to calculate the concentrations of metal species in the system, including the metal bound to the biotic ligand. In reality, this equilibrium is not always reached in artificially spiked test media at the time of testing, and this should be investigated thoroughly when a BLM is developed using literature data or when the BLM is applied on toxicity data reported in scientific literature.

The conceptual part of the Biotic Ligand Model can be considered in terms of three separate components (Figure 8). The first component involves the solution chemistry in the bulk water, which allows prediction of the concentration of the toxic metal species. These chemical speciation computations are standard and can be performed with any of the several models that exist; many models have been proposed. Di Toro et al. (2001) used WHAM version 5, in combination with the modified version of CHESS. WHAM was selected as this model has been calibrated to multiple data sets for the dissolved organic matter complexation, the model is fully described and the computer code is available. A second component involves the binding of the toxic metal species to the BL. The final component is the relationship between metal binding to the BL and the toxic response.

As mentioned before, the enormously varied nature of DOM in aquatic systems presents major challenges in predicting metal complexation (Paquin et al., 2002). To counter this uncertainty, the BLM framework of Di Toro et al. (2001) uses chemical equilibrium computations made with CHESS. It includes the simultaneous solution of a standard set of metal-inorganic matter reactions

**Figure 8:** Schematic overview of the Biotic Ligand Model for copper. Dashed lines represent speciation reactions (modelled by WHAM; Tipping, 1994); solid lines represent binding to the biotic ligand (which may be toxic action sites or transport sites). The free copper ion ( $\text{Cu}^{2+}$ ) forms complexes with inorganic ligands such as  $\text{OH}^-$  and  $\text{CO}_3^{2-}$  (other ligands not shown). The concentration of  $\text{OH}^-$  and  $\text{CO}_3^{2-}$  is determined by pH and alkalinity.  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$  also form complexes with dissolved organic carbon (DOC).  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{H}^+$  (also determined by pH) compete with copper for binding sites on DOC.  $\text{Cu}^{2+}$ ,  $\text{CuOH}^+$  and  $\text{CuCO}_3$  bind to the BL, and the Cu-concentration bound to the BL determines the toxic effect. The latter concentration is assumed to be constant for a given effect size (e.g., 50% mortality).  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{H}^+$  can compete with copper for BL-sites, thus decreasing the amount of Cu bound to the BL (and thus the toxicity) at a given dissolved copper concentration. This concept was first formulated in the Free Ion Activity Model (Morel, 1983) and the Gill Surface Interaction Model (Pagenkopf, 1983). Binding affinities of  $\text{Cu}^{2+}$ ,  $\text{CuOH}^+$ ,  $\text{CuCO}_3$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{H}^+$  are defined by  $K_{\text{CuBL}}$ ,  $K_{\text{CuOHBL}}$ ,  $K_{\text{CuCO}_3\text{BL}}$ ,  $K_{\text{CaBL}}$ ,  $K_{\text{MgBL}}$ ,  $K_{\text{NaBL}}$  and  $K_{\text{HBL}}$ , respectively.





in combination with the metal-organic matter reactions that were developed for WHAM, Model V (Tipping, 1994). This formulation of WHAM includes a detailed representation of DOM chemistry, including proton and metal binding on numerous heterogeneous binding sites and a continuum of binding affinities suitable for a range of metal concentrations that are typical for natural waters. The model also considers competition with protons and calcium on metal binding sites within DOM. Due to its input parameters and complexity of assumptions regarding organic matter complexation of metals, WHAM V is more sophisticated than other speciation models that have been used within a BLM-framework (e.g., MINEQL+ in the Ag-BLM by McGeer et al. (2000)) (Paquin et al., 2002).

With regard to the speciation calculation, none of the existing models has the capability to fully model the metal complexation in terms of the full heterogeneity of natural waters, which include colloids, particulates and complexing cations to DOC like Al and Fe, as well as dissolved ligands (e.g., EDTA). The speciation module in the BLM, however, appears to be the most suitable available computer code for assessing metal speciation in natural waters, and it continues to undergo refinement and further testing (Paquin et al., 2002). It should be noted, however, that the usefulness of the BLM in waters with a DOC-composition that deviates from DOC in natural waters (e.g., porewater, STP-effluents) has not been evaluated yet. It is possible that the modelled speciation in these waters is not in line with actual speciation, resulting in a poor predictive capacity of the BLM for these waters. This should be further investigated in field validation studies. Future BLM developments will undoubtedly benefit from improvements in the detection techniques for direct measurements of low free ion metal concentrations (<< µg/l, e.g. more sensitive ion-selective electrodes). Availability of such data will improve the modelling of metal speciation and binding to DOM, biotic ligands and other sites that play a role with regard to the manifestation of toxic effects.

The biological compartment of the BLM assumes that the biotic ligand occurs on a biological surface (e.g., the gill) and that metal binding can be characterized as a surface adsorption process. Competition is assumed to occur between the toxic metal and  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{H}^+$  (McGeer et al., 2000; Santore et al., 2001; Schwartz and Playle, 2001; De Schamphelaere and Janssen, 2002) for

binding sites on the biotic ligand. Other potential competing cations like potassium have also been investigated, but they did not influence Cu- or Zn-toxicity to daphnids or algae (De Schamphelaere and Janssen, 2002; Heijerick et al., 2002a,b). The competition between the metal ion and other constituents in the aquatic phase determines the amount of metal that binds to the biotic ligand (Di Toro et al., 2001).

The toxicity of metals to organisms is assumed to occur as the result of the toxic metal species reacting with the physiologically active binding sites at the site of action. This is represented as the formation of a metal-biotic ligand complex. For fish, the biotic ligand is either known or suspected to be the sodium or calcium channel proteins in the gill surface that regulate the ionic composition of the blood. For other organisms, it is hypothesized that a biotic ligand exists and that mortality can be modelled in a similar way. Thus, the BLM replaces the fish gill as the site of action with a more generally characterized site, the biotic ligand. The reason for this replacement is to emphasize that this model should be applicable to other aquatic organisms than fish.

Different methodologies have been proposed to estimate or derive the conditional binding constants of metals and competing cations to the BL. Binding constants for metals, protons and other cations have been derived from gill-loading experiments with juvenile rainbow trout (Bury et al., 1999a,b; Galvez and Wood, 1997; Playle et al., 1992, 1993a,b; Janes and Playle, 1995). Calibrating existing models to toxicity data proved to be of practical utility in cases where the mechanism to metal toxicity is less understood or in cases where it may not be possible to directly evaluate metal accumulation at the biotic ligand (i.e., in very small invertebrates). De Schamphelaere and Janssen (2002) proposed and applied a mathematical approach that allowed the derivation of binding constants from univariate toxicity test series. Binding constants that were obtained with this method were in line with previously reported values that were found with other approaches (De Schamphelaere and Janssen, 2002, 2004; De Schamphelaere et al., 2002; Heijerick et al., 2002a,b).

The similarity among constants for the same metal or cation but different organisms suggests that the mechanisms for binding to the BL are similar for different organisms (Paquin et al., 2002) and supports the validity of the cross-reading approach for SSD normalization. Another important issue of BLM development is the validation and fine-tuning of the BLM algorithms through experiments in natural waters. This indeed provides an important check of the capacity of the model to predict ecotoxicity response in field waters from lab waters, and as such addresses part of the uncertainty related to the lab-to-field extrapolation. Existing BLMs have proved their predictive capacity in natural waters that are covered by the physicochemical boundaries for which the models were developed. On the other hand, it remains unclear to what extent these models are useful under more extreme conditions (very low/high hardness and/or pH, high levels of other ions like Fe, Al) without the need to adjust the speciation-component of the BLM or the binding properties of the biotic ligand.

With regard to the recent development of chronic BLMs, it should be noted that strong binding sites on DOM, for instance, may not be saturated at these low exposure Me-concentrations and could therefore exert a controlling influence over metal speciation, whereas this is not the case at acute exposure levels. The use of multiple binding site speciation models that allow competition for other cations (e.g., WHAM-CHESS) is more likely to be able to account for low-concentration higher-affinity binding sites and should therefore be used in chronic BLM-development. In this light, other high-affinity ligands for metal complexes in natural waters should also be taken into account. Bianchini and Bowles (2002), for instance, have discussed the need to include reduced sulfur ligands, including inorganic sulfide species and thiols, into the speciation calculation of the BLM. These types of complexants are not considered in the speciation compound of the BLM and may account for some of the remaining variation between observed and predicted chronic effect levels. It might also be desirable to further investigate the degree to which metal species other than the free ion ( $\text{Me}^{2+}$ ) can exert toxicity. The toxicity and incorporation into the BLM of inorganic metal complexes has already been established for copper ( $\text{Cu}(\text{OH})^+$  and  $\text{CuCO}_3$ ; De Schamphelaere et al., 2002) and silver ( $\text{AgCl}$ ; Paquin et al., 1999).

BLM development efforts should also profit from the consideration of new physiological information as it becomes available, as efforts proceed to extend the BLM beyond the organisms they currently have been developed for. The current formulation of the BLM, for instance, assumes that metal uptake across the gill is slow and therefore the BL-surface achieves equilibrium with the surrounding environment. Recent studies with Cd, however, indicate that this may not always be the case (Tran et al., 2001, 2002). A further improvement of BLM-predictions may be achieved by taking this phenomenon (i.e., non-equilibrium conditions) into consideration. In addition, the nature and dynamic properties of the biotic ligand needs to be considered in future BLM-conditions, as the BL is a part of a living organism that is very likely to change in response to environmental perturbations.

Finally, two other aspects that might contribute to the remaining variability between observed and BLM-predicted toxicity are dietary exposure and ageing. De Schamphelaere and Jansen (2004) recently demonstrated that increased copper levels in algal food had a beneficial effect on Cu-toxicity. The importance of ageing is related to one of the main assumptions – i.e., that the system is in equilibrium. When the metal under investigation is added, the system will need some time to come to equilibrium. In many cases, however, the test medium is not aged and tests are performed immediately after the metal has been added, which could influence the outcome of the BLM. Therefore, by taking these processes into account, future refinements of the BLM may further reduce the difference between observed and predicted effect levels. It should also be noted that metals do not occur in isolation in the environment but are present as a mixture. Mixture toxicity is not yet part of BLM work but should be developed in the future.





## 4.2 Speciation/bioavailability of metals and metal compounds in the sediment compartment

Trace metals discharged into aquatic ecosystems are most likely to be scavenged by particles and removed to sediments. Once associated with surface sediments, metals are subjected to a multitude of *transformation* reactions occurring during *early sediment diagenesis*, leading among others to mineralization and the formation of secondary minerals, binding to different sediment fractions (sulfides, organic carbon, Fe-Mn (oxy) hydroxides etc.).

It is no surprise therefore that, at the moment, no clear relationship has been established between measured total concentrations of contaminants in sediments and their potential impact on aquatic life. As a result, comparing predicted environmental concentrations expressed on a dry or wet weight basis with an established safety level (PNEC) has the potential to result in an under- or overestimation of the associated risk. It is clear that for a risk assessment purpose and, in particular, the derivation of Sediment Quality Criteria, procedures based solely on total concentrations have to be improved by taking into account several lines of evidence that should be compared in a weight of evidence approach. This type could include:

- Evaluation of benthic community indices;
- Information on pore water and overlying water chemistry;
- Toxicity assays that include (if possible) both dietary and waterborne exposure to relevant sensitive organisms;
- Results of dynamic multi-pathway bioaccumulation modelling; and
- Information on relevant binding phases in sediments (SEM/AVS, iron and manganese oxyhydroxides, organic matter, silicates and carbonates).

The principles and concepts of a weight of evidence approach will not be discussed further since a specific fact sheet will address this issue. Here the focus will be on the assessment of the bioavailable fraction of metals and metal compounds present in the sediments. This involves assessing binding to reactive sulfides, organic carbon and potentially other reactive ligands and surfaces.

### 4.2.1 Bioavailability of sulfide binding metals

#### 4.2.1.1 SEM-AVS concept

In anoxic sediments, sulfide produced by sulfate reduction reacts with  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  to form iron and manganese sulfide solids, including amorphous iron sulfide, mackinawite, greigite, pyrrhotite, troilite, pyrite and pink and green manganese sulfides (Wang and Chapman, 1999). Although pyritic sulfide phases are both abundant and reactive towards trace metals, iron monosulfides, quite often referred to as 'Acid Volatile Sulfides' (AVS), are considered to be a more reactive sulfide pool. According to Diaz et al. (1998), AVS is the summation of amorphous FeS, mackinawite (FeS) and greigite ( $\text{Fe}_3\text{S}_4$ ). Acid volatile sulfide is, however, in the first place an operational defined parameter indicating those sulfides that are readily extracted by the cold extraction of sediment in approximately 1 M HCl acid.

Another term that is used in conjunction with AVS is SEM. Simultaneously Extracted Metal can be defined as the metal that is simultaneously extracted under the same conditions under which the AVS content is determined. If multiple metals are present it is necessary to use the term total SEM ( $\Sigma$  SEM). The equivalent release of sulfide (AVS) and metal, however, does not necessarily mean that the metal is bound by sulfide alone. SEM refers to the metal associated with the sulfides and any other metal-bearing phase that is extracted in the cold HCl extraction used for AVS analysis (Allen et al., 1993). For example, metal sorbed to iron oxides and particulate organic carbon will also be extracted.

Di Toro et al. (1990, 1992) have proposed an SEM/AVS Model based on the recognition that AVS is a reactive pool of solid phase sulfide available to bind with metals, forming insoluble metal-sulfide complexes that are non-bioavailable. The basic concept behind the AVS approach is that most metals have higher solubility products than most iron and manganese mono sulfides (except for pyrite) and hence can displace iron from its sulfide complex on a mole-to-mole basis, forming insoluble sulfide complexes with minimal biological availability (Ankley et al., 1996):

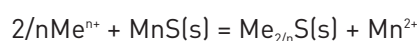
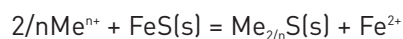
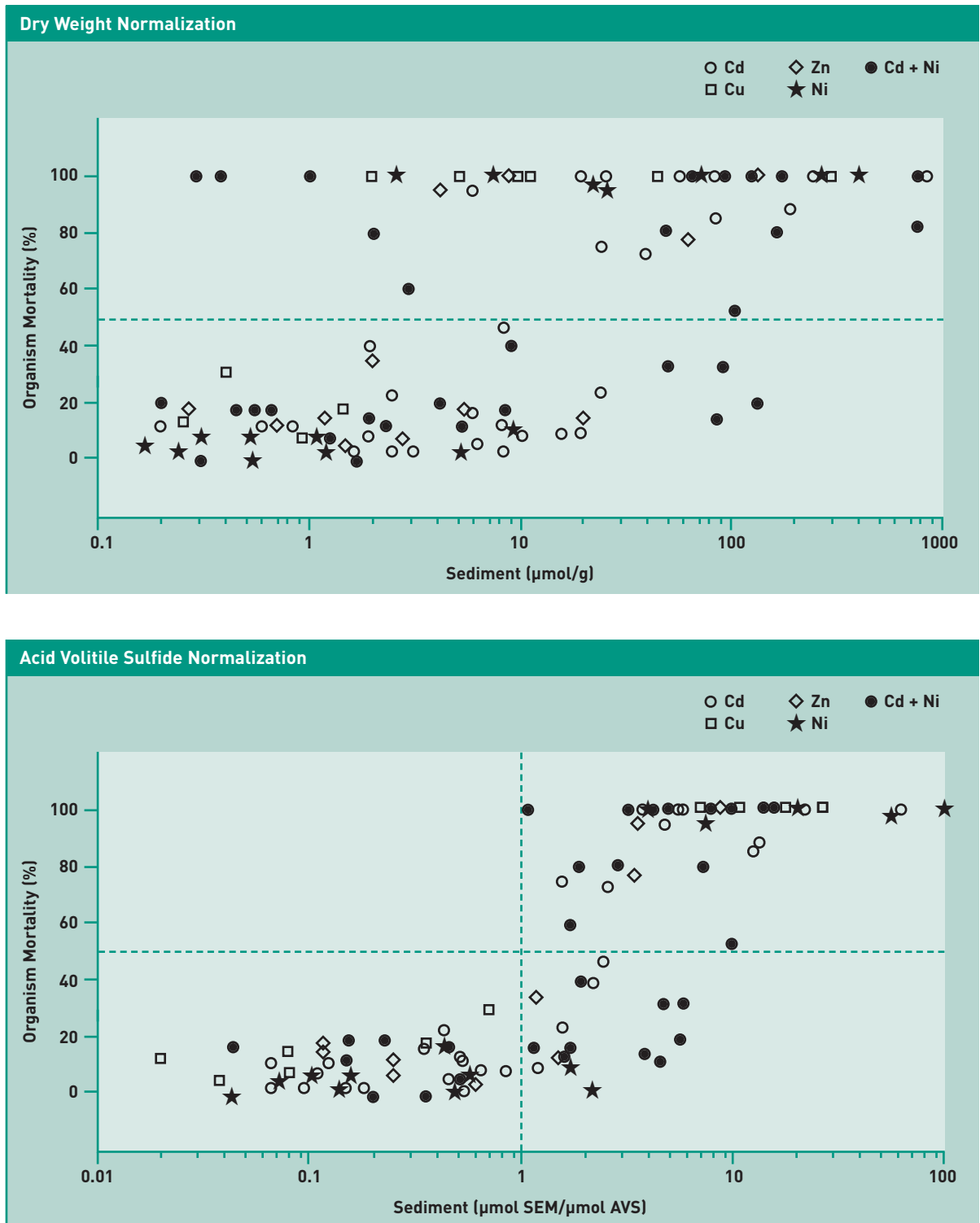


Figure 9: Effect of AVS normalization on the predictive capacity of sediment toxicity





If all the metal in sediment is in the form of  $Me_{z/n}S(s)$  (i.e., AVS in excess), then the free metal ion activity is controlled by dissolution of  $Me_{z/n}S(s)$ .

The SEM-AVS Model predicts that when the measured AVS concentrations exceed the concentration of SEM (SEM/AVS ratio smaller than 1), the pore water levels of free metal ions should be very low, resulting in the prediction of no toxicity. When SEM exceeds AVS, higher pore water concentrations of the metal(s) are predicted, resulting potentially in toxicity. It should be noted that the SEM-AVS Model was not intended to predict metal-related toxicity. In this regard, using the difference between the molar concentrations of SEM and AVS (SEM-AVS) instead of the SEM/AVS ratio can provide important information. The SEM-AVS difference gives insight into the extent of additional binding capacity, the magnitude by which AVS binding has been exceeded and (when organism response is considered) the potential magnitude of importance of other metal-binding phases (Hansen et al., 1996). At a molar SEM-AVS difference  $<0$ , no effects are expected. Recently the existing AVS method has been adapted by taking also the partitioning of metals to sediment organic carbon into account (Di Toro et al., 2004) in order to predict not only the lack but also the onset of metal toxicity in spiked and field-contaminated sediments.

In applying the SEM-AVS Model for a specific metal it has to be taken into consideration that metals are acting in a competitive manner when binding to AVS. Acknowledging the existence of competitive displacement kinetics, the SEM-AVS Model can be made metal-specific. The procedure that is used is to assign the AVS pool to the metals in the sequence of their solubility products. For example, ranked from the lowest to the highest solubility product the following sequence is observed for these five metals:  $SEM_{Cu}$ ,  $SEM_{Pb}$ ,  $SEM_{Cd}$ ,  $SEM_{Zn}$  and  $SEM_{Ni}$ , meaning copper has the highest affinity for AVS, followed by lead, cadmium, etc. until the AVS is exhausted. The remaining SEM is that amount present in excess of the AVS.

To be specific, let  $\Delta \{SEM_i\}$  be the excess SEM for each of the  $i^{th}$  metals. The least soluble metal sulfide (of the five metals considered above) is copper sulfide. Thus if the simultaneously extracted copper is less than the AVS ( $\{SEM_{Cu}\} < \{AVS\}$ ), then essentially all of it must be present as copper sulfide with no additional  $SEM_{Cu}$  present, such that  $\Delta \{SEM_{Cu}\} = 0$ . The remaining AVS binding pool is  $\Delta \{AVS\} = \{AVS\} - \{SEM_{Cu}\}$ . This computation

is repeated next for lead and cadmium because these are the next least soluble sulfides. Suppose, as an example, that unlike copper and lead the simultaneously extracted cadmium is not less than the remaining AVS  $= \Delta \{AVS\} = \{AVS\} - \{SEM_{Cu}\} - \{SEM_{Pb}\}$ . Hence, only a portion of the simultaneously extracted cadmium is present as cadmium sulfide and the remainder is present as excess SEM. Because the AVS has been exhausted by the cadmium in this example, the remaining two metals, zinc and nickel, would all be present as excess SEM.

For example, suppose 1 mole of AVS is present, 0.3 moles of  $SEM_{Cu}$ , 0.4 moles of  $SEM_{Pb}$ , 0.5 moles of  $SEM_{Cd}$ , 0.2 moles of  $SEM_{Zn}$  and 0.1 moles of  $SEM_{Ni}$ . Then  $\Delta \{SEM_{Cu}\} = \Delta \{SEM_{Pb}\} = 0$ . The remaining AVS  $= \Delta \{AVS\} = \{AVS\} - \{SEM_{Cu}\} - \{SEM_{Pb}\} = 1 - 0.3 - 0.4 = 0.3$  moles. Since the  $\{SEM_{Cd}\}$  exceeds the remaining AVS,  $\Delta \{SEM_{Cd}\} = \{SEM_{Cd}\} - \Delta \{AVS\} = 0.5 - 0.3 = 0.2$  moles. Because all AVS has been exhausted  $\Delta \{SEM_{Zn}\} = \{SEM_{Zn}\} = 0.2$  moles and  $\Delta \{SEM_{Ni}\} = \{SEM_{Ni}\} = 0.1$  moles. It is clear from this calculation that this approach is a conservative one, since in this case only a portion of AVS is allocated as a binding phase for nickel.

The literature is replete with studies that investigate the effectiveness of the SEM-AVS method. The model appears useful for predicting the absence of metal toxicity in sediments in short-term toxicity tests (Ankley et al., 1994, 1996; Di Toro et al., 1990, 1991; Casas and Creclius, 1994; Pesch et al., 1995; Berry et al., 1996). Also, chronic toxicity tests support the use of the SEM-AVS Model as a predictive tool for sediments that are unlikely to be toxic, but the evidence is less exhaustive. A total of six full life cycle and colonization toxicity tests were conducted in the laboratory and field using sediment spiked with individual metals and metal mixtures (Hare et al., 1994; De Witt et al., 1996; Sibley et al., 1996; Hansen et al., 1996; Liber et al., 1996; Boothman et al., 2001).

The weight of evidence shows accurate prediction of no toxicity in more than 90% of the studies where excess AVS was present. In a few separate cases, toxicity was observed in field experiments while according to the SEM-AVS Model no metals should be bioavailable. For example in Liber et al. (1996), in 2 out of 17 freshwater field sites toxicity was observed when excess AVS was present. This does not automatically imply that the SEM/AVS Model is flawed. One possibility is that the observed toxicity

is not caused by metals but was due to the presence of other contaminants that have not been measured. It could also be partly due to sampling resolution in which the mean measured SEM-AVS values may not always reflect what a benthic organism may actually 'see'. The bulk of evidence, however, in these field sediments is supportive of the SEM/AVS Model as illustrated in Figure 9.

As shown in Figure 9 the SEM/AVS concept does reasonably well in predicting the absence of toxicity relative to total metal concentrations where no predictive ability is shown for toxicity in sediments. Testing of 125 different field-sediments showed the AVS approach accurately (99.2%) predicted the absence of toxicity (Hansen et al., 1996).

The applicability of the SEM/AVS Model has recently also been evaluated by Shine et al. (2003). Receiver Operating Characteristics (ROC) curves were used to compare different approaches/models that estimate the toxicity of metals in sediments. The focus of the evaluation was on the extent to which a method was able to correctly classify a toxic sample as toxic and a non-toxic sample as non-toxic. ROC curves were constructed by using acute toxicity data from 357 samples chosen from eight sources, including freshwater and marine sediments. The species tested were *Hyalella azteca*, *Chironomus riparius*, *Neanthes arenaceodentata*, *Capitella capitata*, *Lumbriculus variegatus*, *Helisoma* spp., *Ampelisca abdita* and *Chironomus tentans*. The results on the SEM/AVS Model evaluation showed that this approach has a very high sensitivity (96%) – i.e., the extent to which a model correctly classifies a toxic sample as toxic and is therefore protective of the environment. Next to sensitivity, both the negative and positive predictive capability was examined. From this analysis it is clear that the SEM/AVS Model provides an adequate negative predictive power of 97% but provides low positive predictive power of 55%. Because the latter is the likelihood that a sample exceeding the threshold is in fact toxic, it means that in a large number of cases exceeding the SEM/AVS ratio does not result in any observed toxic effects. This is not surprisingly since both the SEM/AVS threshold of 1 and SEM-AVS threshold of zero are not intended to predict toxicity but to indicate when absence of toxicity can be expected.

It should, however, be noted that most of the tests validating the AVS Model were done using spiked sediments with short equilibration times in the laboratory. It is clear that whether the measure is toxicity or contaminant uptake (see section 4.2.2.2), both short equilibration times and high spiked metal concentrations in sediments will accentuate partitioning of metals disproportionately to the dissolved phase and increase the probability of exposure or toxicity from dissolved metals to levels that are not environmentally realistic (Lee et al., 2004). This makes it more uncertain to use results of laboratory-spiked studies in determining the partitioning of metals to different binding phases of the sediment in the field where the metals are introduced gradually and equilibrate over longer time horizons and have concentrations that are not so extreme.

The results of Lee et al. (2004), however, generally support the utility of AVS normalization for predicting no acute toxicity of animals exposed to metal-contaminated sediments when SEM-AVS is less than zero. It should, however, be recognized that at the moment it is not possible to preclude unambiguously other routes of uptake, including exposure to metals via diet (Griscom et al., 2000, 2002), which may become important during chronic exposure (e.g., a fraction of the AVS bound metals may still be available to organisms that ingest sediment particles rather than just feeding from porewater).

Another possible limitation of the model is that some sediment organisms create a micro-oxic environment by bioturbation. The possible consequences for the applicability of the model are discussed in section 4.2.1.2.



#### 4.2.1.2 Applicability of the SEM-AVS concept to bioaccumulation phenomena

Although the SEM-AVS concept has not been developed to explain bioaccumulation phenomena, it is worth evaluating the number of studies on metal bioaccumulation versus sediment metal/AVS relationships to further examine the tenet that AVS controls metal bioavailability. Ankley (1996) performed a comprehensive review of these studies and concluded that the preponderance of them indicated reduced accumulation of metals at sediment metal/AVS ratios of less than 1. Nevertheless, there were exceptions to this general observation, two of which occurred in short-term laboratory experiments with cadmium- or nickel-spiked sediments (Pesch et al., 1995; Carlson et al., 1991). In these studies there appeared to be a linear accumulation of cadmium and nickel body burdens with increasing sediment metal concentrations irrespective of the metal/AVS ratio. Unfortunately, some of these study results are flawed. For example, in the study by Carlson et al. (1991) the organisms were not gut-purged prior to analysis of tissue cadmium. The nickel and cadmium concentration used in the Pesch et al. (1995) study was extremely high (200 and 700  $\mu\text{g/g}$  dry wt., respectively) making it possible that even minimal contributions from residual gut contents or surface absorption could contribute significantly to the total body burden of nickel or cadmium measured in the polychaete (Ankley et al., 1996). Also, as previously mentioned, short equilibration time and high spike concentrations could have favoured exposure via the overlying water.

In more recent work, cadmium, silver, nickel and zinc bioaccumulation (not toxicity) was examined in four types of invertebrates: the filter feeding clam *Potamocorbula amurensis*, the facultative deposit feeding clam *Macoma balthica*, the surface deposit feeding worm (polychaete) *Neanthes arenaceodentata* and the head-down deep deposit feeding polychaete *Heteromastus filiformis* (Lee et al., 2000a,b). Results showed in general that metal concentrations in animal tissue correlated with metal concentrations extracted from sediments (for Cd:  $r^2$  of 0.58 for the clam *M. balthica*, and  $r^2$  of 0.76 for the clam *P. Amurensis*), but less with metal in pore water across a range of reactive sulfide concentrations (0.5-30  $\mu\text{mol AVS}$ ). An additional radio tracer experiment was conducted to directly test the bioavailability of dietary Cd and Ag sulfide. Cd and Ag were in this case coprecipitated with FeS onto glass beads.

After 18 hours the beads were directly fed to the bivalves for 5 minutes. Both bivalves assimilated 14-19% Cd from the metal sulfide particles.

These results seem to question the notion that metal bioavailability in sediments is controlled only by geochemical equilibration of metals between pore water and reactive sulfides and to indicate that feeding behavior and dietary uptake next to porewater may play a role in the bioaccumulation of cadmium, silver, nickel and zinc.

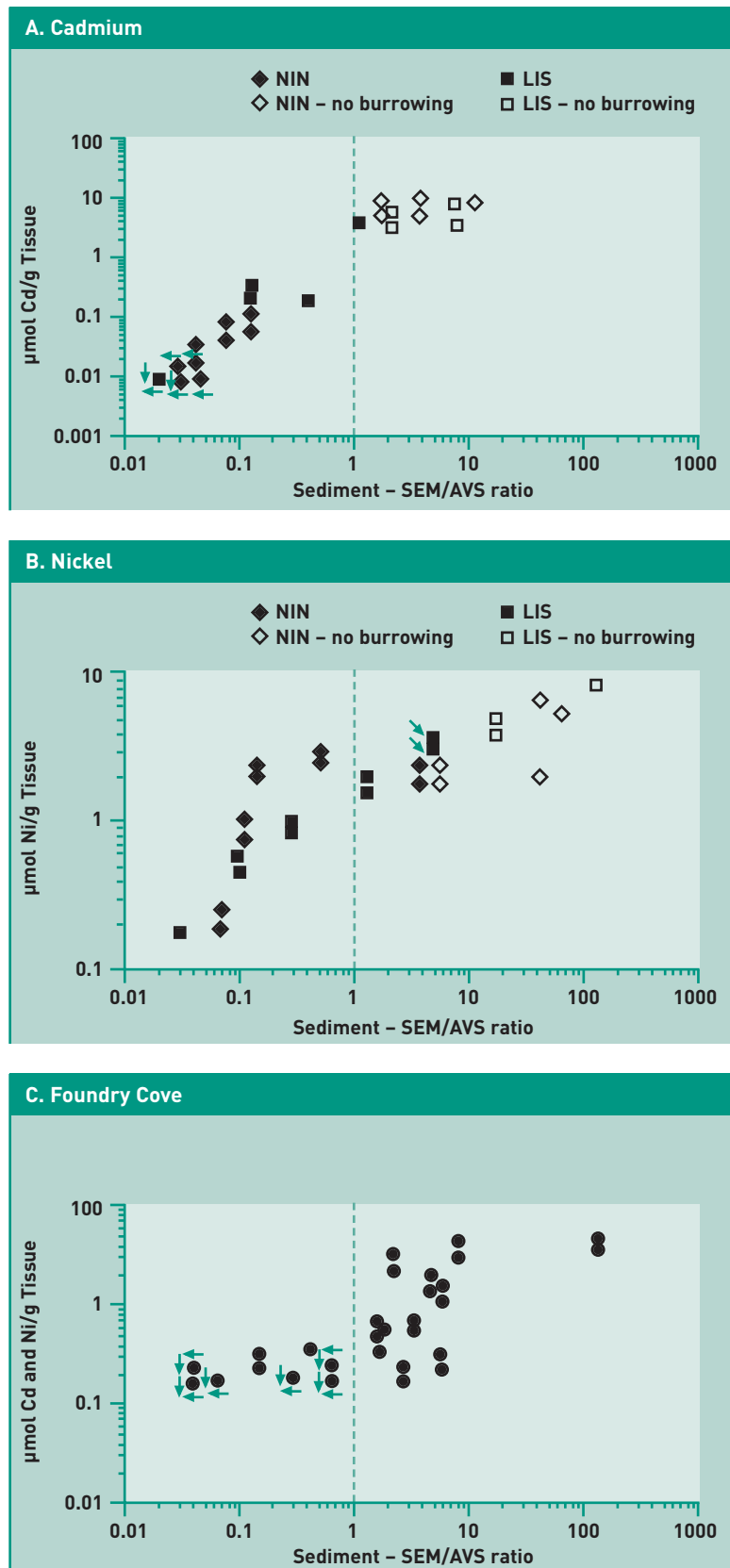
Although it is clear that some contradictory results have been observed among the studies mentioned above concerning the importance of major metal exposure routes (e.g., pore water vs dietary metals), in bioaccumulation phenomena care should be taken in extrapolating the results of these studies to real field conditions. Indeed, most of the laboratory studies just discussed used a protocol that exposed sediment to a spike of dissolved but surface reactive metal and equilibrated the slurry for days before the assay. Metal concentrations in nature, however, are often introduced gradually over a much longer period, resulting in much longer equilibration times in the field.

The importance of equilibration time in spiked sediment bioassays in the partitioning and toxicity of zinc has recently been investigated by Lee et al. (2004). In this study two estuarine sediments with 5 and 15  $\mu\text{mol AVS/g}$  dry wt. were spiked with zinc concentrations between 1 and 40  $\mu\text{mol/g}$  dry wt. and left to equilibrate for 5, 20, 85 and 95 days. Toxicity of the sediments was evaluated using 10 day acute sediment toxicity tests with the amphipod *Leptocheirus plumulosus*. The results showed that both sediment ageing and metal contamination levels are major controlling factors for metal partitioning between sediment particles and the dissolved phase. During equilibration time, AVS concentrations increased with the spiked zinc concentration and the zinc pore water concentrations decreased most rapidly during the initial 30 days and by 11- to 23-fold during the whole 95 day equilibration period.

These results may help to explain some of the discrepancies among the studies regarding routes of metal uptake from contaminated sediments. In this regard, an interesting observation that can be made is that the incompatibility of bioaccumulation behavior and the SEM-AVS concept seems indeed only to be apparent from metal-spiked sediment studies under laboratory conditions in which

**Figure 10:** Bioaccumulation of cadmium and nickel in spiked (two upper graphs) and field sediments (bottom graph) (Pesch et al., 1995)

Accumulation of metal by *Neanthes arenaceodentata* exposed in (A) Cd-spiked sediments, (B) Ni-spiked sediments, and (C) Foundry Cove sediments. The two replicates have been plotted. Oblique arrows indicate Ni-spiked sediment where there was a significant difference in depth of burrowing (see text). In Cd-spiked and Foundry Cove experiments, some samples had SEM-Cd and/or SEM-Ni concentrations below the detection limit. The SEM/AVS ratios for these samples were calculated using the detection limit, and these points were marked with horizontal arrows indicating that the ratio was probably lower than what was plotted. Similarly, some samples of tissue had concentrations of Cd and/or Ni below detection limits; downward arrows indicate these points were plotted at the detection limit for metal tissue.





sediments were only allowed to age for a limited time period (4–16 days). For example, in the study by Pesch et al. (1995) on bioavailability and acute toxicity/bioaccumulation (10 day) of metals to the sediment-ingesting marine polychaete, *Neanthes arenaceodentata* metals were accumulated by worms in cadmium-spiked sediments with SEM/AVS ratios  $<1$ , but this phenomenon was only observed in the laboratory-spiked sediments and did not occur in the contaminated field sediments (Figure 10).

#### 4.2.1.3 Spatial and temporal variability in AVS concentrations and influence of bioturbation

Several studies reported on the dynamic behaviour of AVS in natural systems. Besides the inherent spatial variations observed between different sampling locations, AVS concentrations also differ with depth. Most often the AVS concentration increases with increasing sediment depth and is linked to the redox gradient present in the sediment. This increase may already occur over a small sediment distance (0–10 cm) (Van Den Berg et al., 1998, 2001a). In addition to the spatial component, AVS concentrations tend to be the higher at the end of the summer and during fall and lower in winter and spring (Howard and Evans, 1993; Van Den Hoop et al., 1997; Grabowski et al., 2001). This seasonal variability is not always observed. Van Den Hoop et al. (1997) selected two freshwater sediments to study seasonal variability in AVS and SEM levels. The first sediment was obtained from a small lake, Almere Waterwijk. The second sample was obtained from Kromme Rijn, a small river connected with the river Rhine. Sediments were sampled over 1 year. For the Waterwijk sediment, a nearly constant AVS level of approximately  $1 \mu\text{mol/g DW}$  was observed from February through August, whereas increased AVS levels are found from August till January. This pattern might be the result of de-oxygenation leading to higher reducing activities. For the Kromme Rijn sample, however, no clear relationship between AVS level and sampling time could be found and a nearly constant SEM/AVS ratio was observed. A possible explanation is that the Kromme Rijn is influenced by both seasonal variations and the dynamic behaviour of the overlying water stream. In a similar way, spatial

heterogeneity was put forward to be a better explanation for the observed differences in a study where the vertical distribution of AVS and SEM was considered over different seasons in sediment cores of a recent sedimentation area of the river Meuse (Van Den Berg et al., 1998).

Most of the studies reporting on seasonal variability of AVS have been addressing uncontaminated sediments. For example, the study by Liber et al. (1996) clearly indicated that ZnS is substantially more stable than FeS. The latter was the dominant AVS form in the control sediment that showed a clear seasonal variation. However, the same level of fluctuation was not observed in zinc-spiked sediment, with lowest seasonal fluctuation occurring at the highest zinc levels.

Whatever the underlying mechanisms may be, the seasonal cycle of SEM and AVS in natural sediments is ultimately of interest because variation in SEM and AVS can potentially cause non-toxic sediments to become toxic and vice versa. In other words, can a sediment that is non-toxic during the period of high AVS concentration (SEM  $<$  AVS; e.g., the summer) become potentially toxic during periods of low AVS (SEM  $>$  AVS; e.g., the winter) (Di Toro et al., 1996)? Knowledge of spatial and seasonal variations of AVS and SEM levels is therefore required for a proper application of the AVS concept (Van Den Hoop et al., 1997).

Three interconnected factors may help explain the observed patterns reported in literature with regard to the variation in AVS concentration: (1) diagenetic processes (processes transforming sediments into sedimentary rocks), variations in temperature, oxygen and organic carbon content influencing the microbiological activity (2) the stability of the metal sulfide complex with respect to oxidation and (3) bioturbation.

In some rivers and lakes, stratification<sup>1</sup> due to temperature differences may occur in winter. As a result, oxygen levels may indeed be lower at the bottom of a lake due to this phenomenon. If oxygen levels drop, AVS levels will increase, but a lot will depend on the microbial activity during the anoxic period. Microbial activity is the lowest in the winter period. After spring turnover, oxygen levels will

<sup>1</sup> Different types of stratification may occur (e.g., temperature, salt). In estuarine environments and river systems influenced by saltwater intrusion, stratification may occur due to the salt gradient. In these environments oxygen-free conditions will be present in the deep water layers during fall and summer and hence sulfide levels will be high during these periods.

increase and AVS will be more oxidized. But on the other hand due to the higher temperatures in spring and summer, microbial activity will also increase, yielding a higher sulfate reduction rate. The net result is that AVS concentrations tend to be generally higher at the end of the summer and during fall and lower in winter and spring (Table 2).

The observed vertical gradient in sediment AVS is mainly caused by the oxidation of AVS near the sediment/overlying water interface. A factor that may contribute to this is sediment bioturbation (De Witt, 1996), which may bring oxygen to deeper sediment layers due to the burrowing activity of the sediment organism. Peterson et al. (1996) used different densities of the burrowing oligochaete *Lumbriculus variegatus* in a series of laboratory experiments to evaluate the effect of bioturbation on the oxidation of AVS and subsequent bioavailability of cadmium and zinc spiked into freshwater sediments. They showed that the burrowing activity of oligochaete worms significantly reduced AVS concentrations in surficial sediments in a density-related manner and resulted in elevated interstitial water concentrations of Cd but surprisingly not for Zn, for which they have no explanation.

It is relevant to evaluate how the existence of micro-oxic environments (both at the surface sediment layer and in the burrows of some sediment species) can influence the applicability of the SEM-AVS Model.

In those cases where both oxic and anoxic sediments are present, the aforementioned studies demonstrate clearly that the SEM-AVS Model predicts the absence of toxicity on a consistent basis. A possible explanation for these observations is the fact that the aerobic layer at the sediment surface or in oxygenated burrows is rather thin, typically of the order of only a few millimeters to a few centimeters in thickness (Carlton and Klug, 1990; Hesslein, 1976; Statzner et al., 1988; DiToro et al., 1991), so the exposure is still largely driven by the anaerobic chemistry. Since the oxidation process is often relatively slow (see section 4.2.1.4), the rate of conversion of insoluble metal sulfide to dissolved metal in the pore water occurs slowly but might still be important in environments that are not very dynamic. On the other hand, any surficial layer metal sulfide that becomes dissolved in the pore water as a result of metal sulfide oxidation will not simply build up in the pore water and remain there. Rather, it will be subject to diffusion from the pore water into the overlying water as it is produced. Given that the aerobic layer is quite thin, this diffuse flux will tend to offset any increase in pore water metal levels that occur as a result of the oxidation process. Furthermore, pore water metal concentrations will not necessarily be chemically available to benthic organisms, since any metal that is present in the pore water has the potential to form non-bioavailable metal complexes with other pore water ligands, thereby further reducing the potential for toxicity.

**Table 2: Measured AVS concentrations in European freshwater sediments**

Country	Sampling location	Sampling date	AVS (µmol/gDW)
Belgium	Bihain (river)	11-2000	3.0
		04-2001	3.5
Belgium	Someraïn (river)	11-2000	40.0
		04-2001	2.5
The Netherlands	Ankeveen (river)	11-2000	25.8
		05-2001	4.3

Source: EUROECOLE (2001)





#### 4.2.1.4 Remobilization of metals bound to AVS: effects of oxidation and resuspension

Besides spatial and temporal variations, other factors may influence the levels of AVS concentrations found in the sediment compartment. The most prominent factor that can play a role in the decrease of AVS concentration, and that may subsequently lead to a remobilization of the metals bound to the sulfide fraction, is an increase in redox potential (oxidation) that may occur during dredging events or even in the case of a sediment organism oxygenating its burrow.

The transient nature of AVS depends on the nature of the metal-sulfide complex. Most of the studies available evaluating seasonal or spatio-temporal variations of AVS have addressed uncontaminated sediments and were subsequently looking at the dynamics of FeS that is relatively labile. The oxidation of iron sulfide in sediments cannot be taken as indicative of the oxidation of other metal sulfide complexes, which are more stable (e.g., zinc or cadmium sulfide) (Peterson, 1996). Iron sulfides are more susceptible to oxygen diffusion from the overlying water than other metal sulfide complexes.

Di Toro et al. (1996a,b) investigated the kinetics of FeS and CdS oxidation and showed that the oxidation of sediment AVS appears to be biphasic, which may indicate a more resistant component present as well as a reactive component similar to the synthetic FeS used in their experiments. These observations are consistent with the results of recent studies. Simpson (1998) demonstrated that while FeS and MnS are labile and rapidly oxidizable phases, CdS, CuS, PbS and ZnS are kinetically stable for several hours. Sundelin and Erikson (2001) provide further evidence that can reduce the concern on remobilization for other metal sulfides. They showed that after long-term oxygenation of sediment cores (3 to 7 months), Cd, Zn and Cu remain comparatively unavailable. As alluded to above, these observations can partly be explained by the higher stability of the cadmium copper and zinc sulfides with regard to oxidation than iron sulfides, but the long-term stability is suggesting that other ligands in addition to AVS are important for metal bioavailability. Buykx et al. (2000) showed that aeration of sediments during 3 weeks hardly affected the speciation of Ni, Cu and Pb. Zn and Cd were released as AVS levels decreased but were subsequently bound as carbonates or adsorbed to other binding phases. This is consistent with the findings of Mahony et al.

(1996) and DiToro et al. (2001) regarding metal binding to organic carbon in sediments, but also adsorption to several other major other components (e.g., iron and manganese hydrous oxides) present in sediment solid phases can influence the distribution of metals. Zhuang et al. (1994) investigated the effect of aeration on cadmium bioavailability in sediments in a series of lab aeration experiments in batch reactors during periods of approximately one month. During aeration, the concentrations of metals associated with AVS and with pyrite decreased. At the same time there were increases in the concentrations of hydrous iron and manganese oxides, and these materials became increasingly more important in the binding of cadmium. Following the aeration, more than 50% of the cadmium was associated with the extractable iron and manganese components and approximately 2% of the cadmium released during the oxidation of AVS entered the liquid phase. However, in the case of highly contaminated sediments, this 2% release may still be significant.

There is also a potential concern that resuspension of sediments (for example, in the event of dredging) could result in a potential increase in dissolved concentrations of metals in the surface water, primarily related to environmental conditions promoting the shift of trace metals from the particulate state to the dissolved state – e.g., by oxidation of reduced phases.

Since dredging activities are typically intermittent processes in which increased turbidity levels already quickly return to the natural background situation after 30–45 minutes (Van Parys et al., 2001), it is not expected that remobilization of metals will occur to a large extent. Van Den Berg et al. (2001b) collected data on remobilization during a large-scale experimental dredging project conducted under field conditions. The results showed that dredging activities do not notably influence dissolved concentrations of trace metals in the water column. For an overview of similar studies (e.g., Gambrell et al., 1976) supporting these observations, see the sediment review paper by Burton et al. (1991).

These observations could be related to a relatively slow oxidation of metal sulfides or a fast re-supply of liberated trace metals over, for example, freshly formed Mn- and Fe-(hydr)oxides, which together with the organic carbon coating on sediment particles will act as new reactive surfaces having a high affinity for trace metals.

#### 4.2.2 Non-sulfide binding metals: influence of Fe/Mn (oxy)hydroxides, organic carbon and redox potential

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Until now, models or methodologies that can be used for the assessment of bioavailability of metals other than those included in the SEM-AVS concept (Ag, Cd, Cu, Ni, Pb, Zn) have been less developed or seldom applied. Yet the toxicity and bioavailability of these non-sulfide binding metals (e.g., Cr, Co, Hg, Se, V, etc.), that is not determined by the amount of AVS in the sediment layer can be seriously influenced by the presence of organic material, Fe- and Mn-oxyhydroxides and the redox state of the sediment. The incorporation of these concepts may as such have its merits for a realistic assessment of the toxicity of non-sulfide binding metals. These parameters, however, may also affect the toxicity and bioavailability of sulfide-binding metals. Indeed although sulfides have been identified as a main factor for buffering the bioavailability of metals in (anoxic) sediments, toxicity may still not be seen even if the sulfide pool becomes exhausted. This implies the importance of other binding phases, e.g. organic ligands and dissolved/colloidal Fe or Mn oxides (Müller and Sigg, 1990), which in addition contribute to the reduction of metal bioavailability.

#### 4.2.2.1 Metal adsorption and complexation to inorganic and organic ligands

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Trace metal chemistry of oxidized sediments (e.g., at the sediment surface of oxic water bodies) is more complex than that of anoxic sediments. Sulfides dominate the metal speciation processes in anoxic (reducing) sediments (Morel et al., 1975), whereas organic material, carbonates and hydrous oxides of iron and manganese may all compete for the binding of metals in oxidized sediment (Landner and Reuther, 2004). These categories have different behaviours with respect to complexation and remobilization under different environmental conditions (Förstner, 1985). Carbonates, for instance, will hardly be present in the oxidized sediment of low alkalinity freshwater systems (Young et al., 1992). Trace metals can also be associated with other phases of the sediment, like clay particles or carbonate bound (e.g., discrete carbonate minerals co-precipitated with major carbonate phases) (Tessier and Campbell, 1987).

#### *Mn- and Fe-oxides*

Manganese oxide and hydroxide minerals are found in a wide variety of geological settings and are nearly ubiquitous in sediments and soils (Post, 1999). Similar to Fe oxides, Mn minerals typically occur as thin layers of fine-grained, poorly crystalline mixtures. The sorption capacity of (freshly precipitated) Mn oxides is extremely high for a variety of metal cations (Nicholson and Eley, 1997; Murray, 1975). This unusually high adsorption and scavenging capacities of Mn oxide/hydroxide minerals provides one of the main controls of trace metals in aquatic sediments (Young and Harvey, 1992). The presence of only small amounts of Mn oxide minerals in sediment (i.e., a fraction of a weight per cent) can therefore be adequate to control distribution of trace elements between earth materials and associated aqueous systems. As the adsorption of trace metals by hydrous Mn oxides is accompanied by release of protons (H<sup>+</sup>), it has been suggested that the cations are bound into the Mn oxides' atomic structures (Murray, 1975). Mn oxides have been used to recover Ra, Pb and Po from seawater, and it has been shown that they control, together with Fe oxides, the geochemical distribution of several naturally occurring radionuclides like radium (226 and 228 isotopes) and thorium (228 and 234 isotopes) (Todd et al., 1988; Wei and Murray, 1991).



The Fe/Mn oxide component of sediments is an important, even controlling, repository for a wide variety of metals in the majority of these systems. Mn oxide minerals can adsorb or incorporate substantial amounts of Cu, Co, Cd, Zn, Ni, Sn, Pb, Ca, Fe, Ra, Hg, U, Pu, Po, As, Se and Th (Wadsley, 1950; Bacon et al., 1980; McLaren et al., 1985; Takamatsu et al., 1985; Todd et al., 1988; Stahl and James, 1991; Wei and Murray, 1991; Nicholson and Eley, 1997; Nicholson et al., 1997; Farrell et al., 1998; Duff et al., 1999; Dong et al., 2000; Foster et al., 2003; Webb et al., 2004; O'Reilly and Hochella, 2003; Tani et al., 2003). Interaction of these metals with even small amounts of Mn oxides has been reported to decrease the dissolved metal concentration by several orders of magnitude (Jenne, 1968; Bacon et al., 1980; Todd et al., 1988; Wei and Murray 1991; Fuller and Harvey, 2000; Kay et al., 2001).

The ability of ferric hydroxide to adsorb trace metals is characterized in single and multi-adsorbate systems (Patoczka et al., 1998). Metals can be adsorbed both as cations ( $\text{Cr}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ) in neutral to high pH and as anions ( $\text{SeO}_4^{2-}$ ,  $\text{CrO}_4^{2-}$ ,  $\text{VO}_3[\text{OH}]^{2-}$ ,  $\text{AsO}_4^{3-}$ ) in neutral to mildly acid pH. A rough rule of thumb is that one gram of amorphous ferric oxide is capable of adsorbing 0.5 milli moles of ionic material (e.g., trace metal cations, oxyanions of chrome, arsenic or selenium) (Vance, 2002). The sorption behaviour is related to the pH, with each ion having its own optimum pH range for adsorption. The impact of pH on the adsorption efficiency for a number of ions has been summarized by Manzione et al. (1994). For cationic trace metals, the adsorption efficiency increases with pH, while concentration of both sorbate and ferric hydroxide play a secondary role (Farley et al., 1985).

In Lake Baikal, where massive Fe and Mn oxide concretions have been deposited over millennia with little human influence (Müller et al., 2002), it is observed that Mo and Cd are preferentially associated with Mn-rich deposits whereas As is associated with Fe-rich ones. Other metals, like Zn, Cu and Pb, were not correlated with either Fe or Mn oxides. This is more or less confirmed by the findings reported by Young et al. (1992). For selenium, the affinity towards amorphous iron oxyhydroxide is greater than that towards manganese dioxide. The valence state of this element plays also an important role with regard to its affinity for oxides: the adsorption of selenite [ $\text{Se(IV)}$ ] ion to Fe and Mn oxides is stronger than

that of the more toxic selenate [ $\text{Se(VI)}$ ,  $\text{SeO}_4^{2-}$ ] (Balistrieri and Chao, 1990). Peak and Sparks (2002) investigated the binding of selenate to iron oxides and found that this anion forms different surface complexes on iron oxides, depending upon pH, ionic strength and iron oxide structure (e.g., hematite, goethite, amorphous iron hydroxide). The importance of these Fe oxides for assessing metal bioavailability towards benthic organisms has been demonstrated by various authors. Prediction of trace metal levels was improved when the trace metal concentrations extracted from the sediments were normalized with respect to iron (hydrated oxide) (Pb: Luoma and Bryan, 1978; As: Langston, 1980; Cu: Tessier et al., 1983, 1984).

It should be noted that the presence of other, naturally occurring inorganic ligands may affect metal adsorption to Mn and Fe oxides. As a result of competitive adsorption, silicic acid – which is more mobile in the aquatic environment than Fe oxides – may be responsible for an enhanced mobility of chromate in natural systems (Garman et al., 2004).

### Organic ligands

Beside Mn and Fe oxides, metals can also form complexes with organic ligands that are present in the sediment layer. Similar to the chemical reactions that occur between dissolved metal and dissolved organic carbon in the aquatic environment, the free metal ion will form complexes with the carboxylic and phenolic functional groups on the organic molecules, but other groups like amino- and sulfidic groups may also play an important role. The amount of metal that can be complexed by these sediment-associated organic ligands is metal-dependent: Mahoney et al. (1996) found that additional binding to sediment organic carbon of copper and lead could amount to 1–10 and 10–100  $\mu\text{mol/g}$  OC, respectively. For cadmium, on the other hand, this was less than 1  $\mu\text{mol/g}$  OC. According to Jonasson (1977), the binding strength of mercury towards humic/fulvic acids in sediments is stronger than that of sulfide-binding metals, whereas the binding strength of cobalt to these organics is lower compared with this group of metals.

This is in line with Evans et al. (1988), who observed that <sup>60</sup>Co added to sediment is rapidly incorporated into the exchangeable carbonate-bound and oxid-bound fractions, with little <sup>60</sup>Co in the organic and residual fractions. The observation that metals may bind strongly to organic carbon suggests that in some case organic carbon normalization might reduce the variability observed in toxicity levels.

The incorporation of organic carbon into the existing AVS concept has been suggested by Di Toro to predict not only the lack but also the onset of metal toxicity in spiked and field contaminated sediments (Di Toro et al., 2001). In this context it is assumed that toxicity occurs if the excess SEM goes beyond the binding capacity of the organic carbon present in the sediment. Using this information, it was shown that the organic carbon normalized excess SEM can be used to predict toxicity:

$$SEM_{x,oc} = \frac{\sum SEM - AVS}{f_{oc}} \quad (\text{Eq-1})$$

where  $f_{oc}$  is the organic carbon fraction in the sediment.

In order to calculate the metal-specific threshold that produces toxic effects when exceeded, Di Toro et al. (2004) bridged the gap with another toxicity predicting model, the Biotic Ligand Model, in his recent modelling work developing the first step towards a sediment BLM. The *Daphnia magna* BLM was used to compute the LC<sub>50</sub> concentration of metal on sediment particulate organic carbon, which is in equilibrium with the LC<sub>50</sub> in pore water – following the precepts of the Equilibrium Partitioning Model – and in equilibrium with the critical concentration at the site of action (the biotic ligand). From their results it seems that pore water pH is most important. For pH = 8, the approximate value for cadmium is 100 µmol/gOC. Other metals have thresholds that are a magnitude higher (e.g., Zn-SEM<sub>x,oc</sub> = 1,400 µmol/gOC).

#### 4.2.2.2 Influence of redox potential

The valence state under which a metal occurs in the sediment depends on the redox state of that sediment (oxic/anoxic conditions). This valence state affects both the adsorption capacities, as well as the degree of toxicity. For instance, the more oxidized species of selenium (Se<sup>6+</sup>) and chromium (Cr<sup>6+</sup>) are much more toxic than Se<sup>4+</sup> and Cr<sup>3+</sup>, respectively. An important parameter that determines the oxidation state of a metal is the presence of manganese oxides. Mn oxides are some of the strongest oxidants naturally found in the environment (Tebo et al., 2004), and as such, they participate in a wide variety of oxidation-reduction reactions: they oxidize Se(IV) to Se(VI), Cr(III) to Cr(VI) and As(III) to As(V), thereby influencing toxic metal availability by oxidative precipitation or solubilization (Scott and Morgan, 1995; Manceau and Charlet, 1992; Huang, 1991).

As mentioned previously, the bioavailability and toxicity of chromium is mainly determined by its oxidation state. Chromium(VI) is highly oxidized and unstable in reducing and even moderately oxidizing environments (DeLaune et al., 1998; Masscheleyn et al., 1992). Chromium(VI) is also very soluble, whereas Cr(III) has very low solubility at environmentally relevant pH (DeLaune et al., 1998; Barnhart, 1997) and is generally thought to have relatively low toxicity (Wang et al., 1997; Thompson et al., 2002). Based on these findings, the Cr-hypothesis was formulated and validated by Berry et al. (2004), who stated that in a sediment where AVS is present (reduced, anoxic sediments), Cr will solely exist as the non-toxic Cr(III) form.



### 4.3 Speciation/bioavailability of metals and metal compounds in the terrestrial compartment

A number of studies have examined the influence of physicochemical parameters of soils on the ecotoxicity of metals to terrestrial invertebrates and plants. Relationships between ecotoxicity and pH, organic matter content, clay content and cation exchange capacity (CEC) have been detected and, where possible, incorporated into predictive models (Lock and Janssen, 2001; Spurgeon and Hopkin, 1996; Crommentuijn et al., 1997; Van Gestel et al., 1995; Peijnenburg et al., 2000). Secondly, comparison of toxicity data from laboratory-spiked soils with field-contaminated soils revealed that metals were up to one order of magnitude less toxic in the field soils (Lock and Janssen, 2003; Lock et al., 2003; Smolders et al., 2003; Waegeneers et al., 2004).

From these observations, it can be concluded that the variation of metal toxicity in the terrestrial environment can be explained by the soil type and soil characteristics on the one hand and by the processes that reduce metal toxicity under field conditions or increase metal toxicity in laboratory-spiked soils on the other hand. This section discusses current knowledge on bioavailability, the parameters by which this is affected, metal speciation, ageing of metals under field conditions and how to incorporate this information into the risk assessment process for the different phases of the soil compartment: the aqueous and solid phases.

Various authors have reported that the prediction of bioaccumulation and toxicity is improved when the free metal-ion is considered and not the total metal content in the soil (Minnich et al., 1987; Sauvé et al., 1996, 1998; McGrath et al., 1999; Chaudri et al., 2000). Based on these findings, it has been suggested that an assessment of the bioavailability (and toxicity) of a metal in soil should not be based on test results expressed as the total-soil metal concentration, but on the free ion activity of the metal in the aqueous phase or the soil buffering capacity of the free ion activity (Sauvé et al., 1998).

The Free Ion Activity Model of metal-organism interactions was initially developed to rationalize experimental observations and to explain the initial hypothesis that 'uptake, nutrition and toxicity of cationic trace metals was universally governed by the free metal ion activity' (Morel, 1983). The chemical theory of the FIAM of trace metal availability to higher plants has been reassessed by Parker and Pedler (1997). Many solution cultures showed a good correlation between higher plant responses and the activity of the free metal ions in solution, with responses to the presence of soluble metal-ligand complexes being largely indifferent (Checkai et al., 1987; Bell et al., 1991; Parker et al., 1995; Parker and Pedler, 1997; Cheng and Allen, 2001). Various authors have challenged the hypothesis that the activity of the free metal ion accurately predicts metal uptake or bioaccumulation by plants and, hence, bioavailability. Smolders and McLaughlin (1996a,b) found an increased Cd-uptake in Swiss chard when the chloride-content of the nutrient solution increased but the Cd-activity remained constant. The authors suggested that  $\text{CdCl}_2^{2-n}$  species were also phytoavailable or that chloride promotes the uptake of  $\text{Cd}^{2+}$ . A similar effect was reported by McLaughlin et al. (1998a), who found a marginal but significant increase in the plant-shoot Cd-concentration with increased  $\text{SO}_4^{2-}$  concentrations. Uptake of metal complexes by plants has also been suggested in other studies (Smolders et al., 1998; Parker et al., 2001; Zhang et al., 2001).

A second exception to the FIAM is the increased metal uptake when chelate ligands like EDTA or NTA are present in the soil: addition of NTA led to a twofold increase of cadmium in Cd-contaminated soils, whereas the addition of EDTA resulted in an 11- to 63-fold increase in plant cadmium concentration (Wallace et al., 1977). This type of finding clearly indicates that the behaviour of metal ions in the presence of chelators has to be interpreted with caution (Nolan et al., 2003). Chelate-induced increased cation uptake has also been reported for cobalt, copper, lead, manganese, nickel, zinc and various radionuclides (Wallace and Mueller, 1973; Wallace et al., 1973, 1983; Adriano, 1979; Vyas and Mistry, 1983). It has been suggested, however, that the uptake of these EDTA-complexes may not be mediated by the same mechanisms responsible for the uptake of the free metal-ions (Vassil et al., 1998).

Although the FIAM may provide a reasonable basis on which to predict the bioavailability of metals in soils and surface waters in most cases (Tye et al., 2004), the practical application of the FIAM to soil systems is rendered more difficult by the complexity of and the predominant influence of the processes of adsorption/desorption, precipitation/dissolution, uptake/release, and other parameters that buffer and obscure the exchanges between the solid and the solution phases (Harter, 1991b; Sheppard et al., 1992; Luoma, 1995; Jin et al., 1996; Parker and Pedler, 1997; Peijnenburg et al., 1997). An overview of the most important soil parameters that govern metal speciation in the soil is provided in the next section.

#### 4.3.1 Key parameters that determine metal bioavailability in soils

Based on the reported interactions of metals with soil constituents, the primary soil factors controlling the potential bioavailability of metals are soil pH, available charged sites on soil surfaces, clay content, inorganic constituents (e.g.,  $\text{HCO}_3^-$  and  $\text{Cl}^-$ ), soil organic matter, competing cations and rhizosphere (Allen, 2000). Each of these factors determines the ability of a metal to partition from the solid phase to a soluble phase and hence to react with an important receptor site that eventually may cause adverse effects.

##### Soil pH

The pH of the soil is considered the primary soil variable controlling virtually every chemical and biological process in the terrestrial environment. The net metal charge of a metal-complex is determined by the pH, together with its precipitation/dissolution reactions. Moreover, the number of negatively or positively charged binding sites for cations and anions, respectively, is also governed by soil pH: an increase in pH promotes the sorption and removal from the pore water of metal cations (Bohn et al., 1985). The reverse will be the case for metal anions.

##### Cation and anion exchange capacity

The CEC can be considered as a measure of the soil's capacity to adsorb and release cations and is directly proportional to the number of available, negatively charged sites. Its counterpart, the anion exchange capacity (AEC), represents the ability of a

soil to adsorb and release anions and is thus a measure of available positively charged soil surface sites. The following factors determine the CEC:

- clay content: extremely coarse-textured soils have a CEC of approx. 10 mmol/kg, whereas an average CEC for fine-textured soils is 600 mmol/kg (Bohn et al., 1985);
- clay type: the CEC depends on the type of clay (e.g., montmorillonite, kaolinite) and can vary between 20 and 1000 mmol/kg (Bohn et al., 1985);
- organic matter, generally having a CEC of 2000–4000 mmol/kg; and
- soil pH: increase of CEC with increasing pH.

The AEC is mainly associated with amorphous oxides, and decreases with increasing soil pH. Under environmentally relevant conditions, the number of positively charged sites on the majority of soil types is very small and usually negligible.

##### Clay minerals

Clays are soil particles less than 2  $\mu\text{m}$  in size (Miller and Gardiner, 1998) and therefore have higher surface areas relative to sand (20  $\mu\text{m}$  to 2 mm). Since much of the CEC comes from the negatively charged clay surface sites, clayey soils will make cations much less bioavailable than sandy soils.

##### Inorganic soil constituents

Under certain conditions, metals can form precipitates with inorganic soil constituents such as carbonate and phosphate minerals. The solubility and reactivity of these inorganic metal complexes depend on the metal itself, its oxidation state, the pH and the ligand to which it is associated. By its removal from the soil pore water, the precipitated metal becomes less available. Other reaction sites for the sequestration of metals occur on phases such as oxides of Al, Fe and Mn and silicate mineral edge.

##### Organic Matter (OM) content

The main chemical elements of organic matter are carbon (on average: 58%), oxygen and nitrogen. The organic matter in the soil is mainly formed from plant and animal remains in various stages of decomposition, cells and tissues of soil organisms and substances from plant roots and soil microbes (Sumner, 2000). Binding of metals to organic matter takes place at acid functional groups (e.g., carboxylic groups  $\text{R-COO}^-$ ), which have a high affinity to attract metal cations thus reducing their bioavailability.



Most soils have an OM content of less than 10% (Bohn et al., 1985), but this may vary between >1% for sandy soils to almost 100% for peaty soils.

#### Competition with cations and protons

Competition of major cations ( $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,...) for metal binding sites to organic and inorganic compounds in the soil matrix alters the speciation in the soil solution. Additionally, they may also alter the relationship between free metal ion activities in solution and the toxic effect of metals on plant and (soft-bodied) invertebrates (Parker and Pedler, 1997).

In contrast to the effect of reducing pH, which increases metal availability in soil, uptake of metal from solution is reduced as pH decreases, due to the competition of protons ( $H^+$ ) with metals ions for binding to the receptor sites (Tyler and McBride, 1982; Hatch et al., 1988).

#### Metal background concentration

According to the metalloregion concept, adaptation to natural background levels and probably also test conditions may influence the sensitivity towards metals. However, not many studies have explicitly examined the relationship between the metal background concentration in soil and its influence on toxicity.

#### Rhizosphere processes

There are several plant-related processes that may modify the chemistry of the soil and soil solution (Marschner, 1995; McLaughlin et al., 1998b): reduction of ion activity due to plant metal uptake, followed by metal desorption from soil particle; enhanced metal uptake in the rhizosphere due to convective flow of soil solution to the root; plant-induced changes of soil chemistry; excretion of organic ligands by the plant; formation of new plant material (living or dead); changes due to plant-related microbial activity. Currently the understanding of these mechanisms on metal bioavailability is insufficient to be incorporated on a mechanistic basis into predictive toxicity models.

### 4.3.2 Determination of labile metal fraction in soils

Given the current knowledge of the mechanisms of metal uptake by plants and soil organisms, it is evident that use of total concentrations of metals in soil is not a useful predictor of risk. The observation that biological access of metals in the soil compartment principally occurs through soil solution suggests that determination of soil solution concentrations (or activities) of metals would provide the best predictor of risk. However, as uptake of metals causes a depletion of the soil solution, assessment of the pool of solid-phase metal (labile metal), which buffers the solution metal concentration, may be a more appropriate approach.

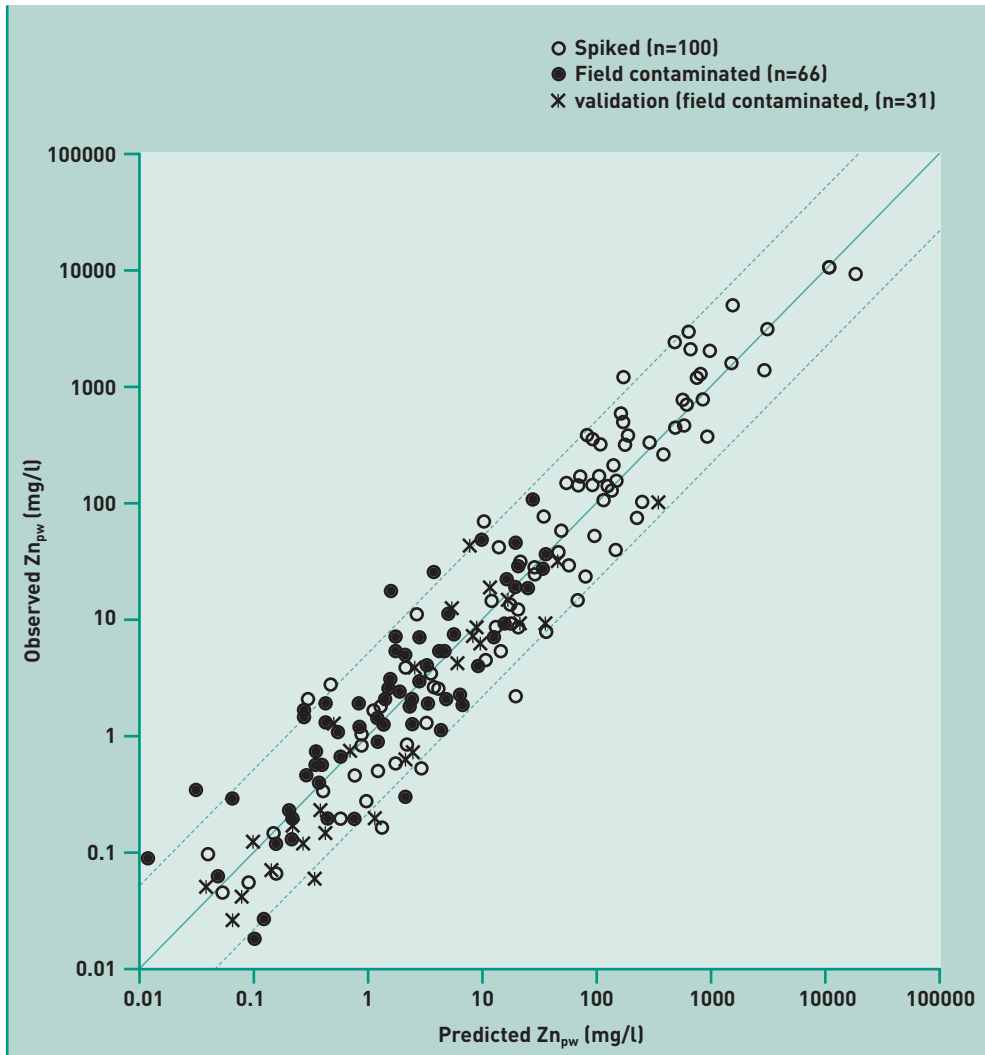
Several methods for estimating or measuring this pool have been described in literature, and sometimes accepted as regulatory standards to control metals in soils (Switzerland: extraction with 0.1 M  $NaNO_3$  (Gupta and Aten, 1993); Germany: extraction with 1.0 M  $NH_4NO_3$  (Prüess, 1997)).

In order to use the labile or free metal soil solution concentration as an input in toxicity-based bioavailability models or to use them for the derivation of a lab-to-field translator, it is essential that these metal concentrations be determined. This can be achieved by estimating the free metal concentrations using chemical equilibrium models, the use of semi-empirical models predicting metal concentrations in soil solution or actual measurements of the free/labile metal fraction. The latter may be preferred over model estimations as the complexity of the different chemical processes that occur in the aqueous and solid phase, and the heterogeneity of the soil matrix, may cause a large uncertainty on the modelled metal speciation.

#### Derivation of the free metal concentration using Chemical Equilibrium Models

Chemical Equilibrium Models can be used to predict trace metal solubility of solutions in equilibrium with pure minerals with good accuracy. Unfortunately, soils are not made of pure minerals, but often have a very complex composition, and the solution may not be at equilibrium. These models do not account for kinetics of precipitation/dissolution of solids or for the slow reaction rates of certain trace metal ligand reactions (Allen et al., 1982; Plankley and Patterson, 1987; Hering and Morel, 1990a,b; Harter, 1991a; Hering, 1995; Ma et al., 1999). In addition, modelling the complexation by

**Figure 11:** Comparison between the observed pore water concentration of Zn ( $Zn_{pw}$ ) and the value predicted with the regression equation (Eq. 3). The solid line represents the 1:1 line and the dotted lines indicate 5 times under- or overprediction (Smolders and Degryse, 2003)







organic matter is even more complicated. Many of the conceptual binding models that have been suggested were designed for aquatic systems: they do not accommodate the very large solid phase component of soils and the accompanying buffer capacity and the complexity of a solid phase in dynamic relation with a transient soil solution that has continuous changing concentrations of mineral and organic particulates. Properly calibrated, however, they can be useful to predict speciation in extracted soil solutions.

Mechanistic 'assemblage' models that predict ( $M^{2+}$ ), such as SCAMP (Lofts and Tipping, 1998) and ECOSAT, which incorporates the NICA-Model (Goody et al., 1995), are attempts to provide some fundamental solutions to the chemical speciation of soil pore waters. However, simpler models that are based on routinely measured parameters in geochemical surveys may have advantages over more rigorously formulated models as they require fewer inputs.

#### Derivation of the free metal concentration using Competitive Adsorption Models

Competitive Adsorption Models are capable of linking the soil solution free metal activity with some very simple soil properties like pH, organic matter, clay content and total metal content. Most of these regressions, however, do not predict the free metal activity but rather the dissolved or "labile" metal concentration (Jopony and Young, 1994; Sauvé et al., 1995, 1997; Janssen et al., 1996, 1997; de Groot et al., 1998; Elzinga et al., 1999). McBride et al., (1997) developed the following semi-empirical equation to relate free metal activity in soil solution to organic matter pH and total soil metal content (Eq-2):

$$-\log a_M = pM = a + b.pH + c.\log([M]_T.[OM]^{-1}) \quad \text{(Eq-2)}$$

where  $a_M$  is the free metal activity,  $[M]_T$  and  $[OM]$  the total metal and organic matter concentrations, respectively, and  $a$ ,  $b$  and  $c$  are constants. Even though the free metal activities were only estimated and not directly measured, they were generally a better indicator of metal toxicity to various biotic endpoints than total metal concentrations in soils. Care must be taken, however, when such simple equations are used in any circumstance and for any soil type: the universality of these semi-empirical equations always needs to be considered.

For example a regression model for the prediction of the pore water concentration of Zn was developed by Smolders and Degryse (2003), using data from both spiked and field-contaminated soils (Eq-3).

$$\log Z_{n_{pw}} = 2.47 + 0.70 \times \log Ca_{pw} - 0.62 \times \log OC + 1.12 \times \log E - 0.82 \times pH \quad \text{(Eq-3)}$$

with  $Z_{n_{pw}}$  the Zn pore water concentration (mg/l),  $Ca_{pw}$  the pore water concentration of Ca (mmol/l), OC the organic carbon content (%), E the (radio)labile Zn concentration (mg/kg) and pH the soil pH. Figure 11 compares observed Zn pore water concentrations and predicted concentrations with Eq-3. In most cases, the difference between observed and predicted was within a factor of 5. This figure demonstrates that although a good correlation is obtained between measured and predicted Zn-concentrations, these semi-empirical relations are currently not capable of accurately predicting metal concentrations in soil pore water.

An advantage to using these semi-empirical models is the fact that bioavailability can be predicted in changing scenarios. Since pH and organic matter content are included in these equations, it is possible to assess not only the actual risk but also the potential risk if these soil parameters were to change as a result of agricultural practices or human activities in general. Including other soil properties such as cation exchange capacity, manganese and iron concentrations and clay content may further improve the prediction of metal concentrations (Nolan et al., 2003).

#### Direct measurement of the labile/free metal concentrations

Since it is difficult to accurately predict pore water metal concentrations by using equilibrium or adsorption models, direct measurement could be a better approach for the quantification of this metal fraction in the soil. There are various analytical procedures for measuring metal speciation in the aqueous phase of soil. Many of these analytical methods determine the labile, bioavailable metal fraction and not the free metal-ion concentration. The most commonly used techniques are:

### *Ion-selective electrode*

The use of this method is only possible for some metals (e.g., Cu, Pb) and implies that only the free ion is considered, so any other labile or bioavailable metal fraction is not considered.

### *Differential pulse anodic stripping voltammetry (DPASV)*

DPASV is a sensitive electrochemical method that can be used to determine the concentration of labile metal (Waller and Pickering, 1990; Pinheiro et al., 1994; Deaver and Rodgers, 1996) and excludes organically complexed metals (Pinheiro et al., 1994).

### *Competitive chelation*

The addition of a strong chelate to soil solutions can also be used for estimating the speciation of free divalent metal ions.

### *Diffuse gradients in thin films (DGT)*

DGT (a layer of chelating resin, overlaid by a layer of hydrogel and a protective filter through which ions can freely diffuse) provides a simple tool deriving the labile metal fraction and for *in situ* kinetic characterization of metal release from its binding agent (ligand or particle) in order to determine the rate and extent to which a metal is supplied to biota. The effective concentrations obtained with DGT may provide a practical and useful surrogate for assessing the risks to soil organisms and plants posed by metals in contaminated soils (Davison et al., 2000; Zhang et al., 2001).

### *Isotopic Dilution Techniques*

The isotopic dilution technique allows partitioning of soil metals into isotopically exchangeable (labile) and non-isotopically exchangeable (fixed) pools, effectively a combined solution- and solid-phase fractionation technique. A recently modified technique used by Hamon et al. (2002b) and Lombi et al. (2003) has regulatory merit in that it can simultaneously provide an assessment as to the stability or reversibility of remediation treatments for metals (Hamon et al., 2002b) and also can be used to measure potential mobilization of metals from fixed to available pools (Hamon et al., 2002a).

### *Exchange resins*

Ion-exchange resins can be used to determine the free metal activity or the relative lability of metals, but the latter does not necessarily provide an estimate of the free metal activity (Lee and Zheng, 1993; Procopio et al., 1997; Esnaola and Millán, 1998).

### *Donnan dialysis*

The speciation of metals in soil solutions has also been determined by means of Donnan dialysis membranes (Dietze and König, 1988; Berggren, 1989; Apte and Natley, 1995).

It is important to recognize that the applied method for collecting a soil solution (e.g., *in situ* collection of tension-cup lysimeters, water displacement and centrifugation, simple batch-type extraction) may also affect the measured metal speciation due to various physicochemical and biological transformation processes.

## 4.3.3 Bioavailability models of metals/metal compounds in the terrestrial compartment

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### *The Biotic Ligand Model*

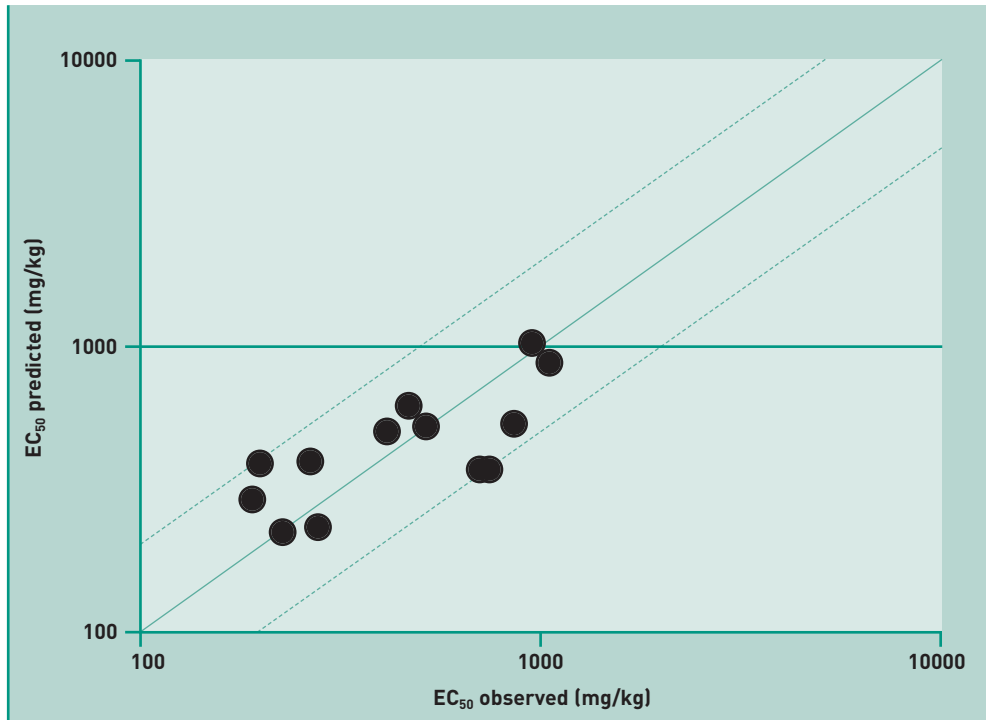
Ideally, in analogy with the water compartment, metal toxicity to terrestrial organisms and plants can be predicted using the Biotic Ligand Model concept. This BLM would be the mathematical integration of the metal interactions with the aqueous-phase ligands affecting its speciation and its subsequent interaction with receptor sites in the soil organisms. The development of a comparable t-BLM that predicts site-specific metal toxicity in soil samples (i.e., incorporation of all bioavailability factors) is only possible if the uptake and toxicity is caused by exposure to metals in the aqueous phase and if the speciation in the aqueous phase can be modelled or measured accurately. The first criteria would assume that ingestion of metal-contaminated soil particles does not contribute to the overall metal toxicity. The latter may be the case for plants and soft-bodied invertebrates but is not applicable for hard-bodied invertebrates, which are primarily exposed through ingestion: it is obvious that the determinants of metal bioavailability must be understood fully if one is to predict the effect of a metal. Risk assessment should incorporate the essential factors that dictate the actual availability of metals. At present, relationships between chemical forms of metals and the bioavailability of hard-bodied organisms may not be sufficiently understood to permit the prediction of uptake or toxicity of the metal using mechanistic models.



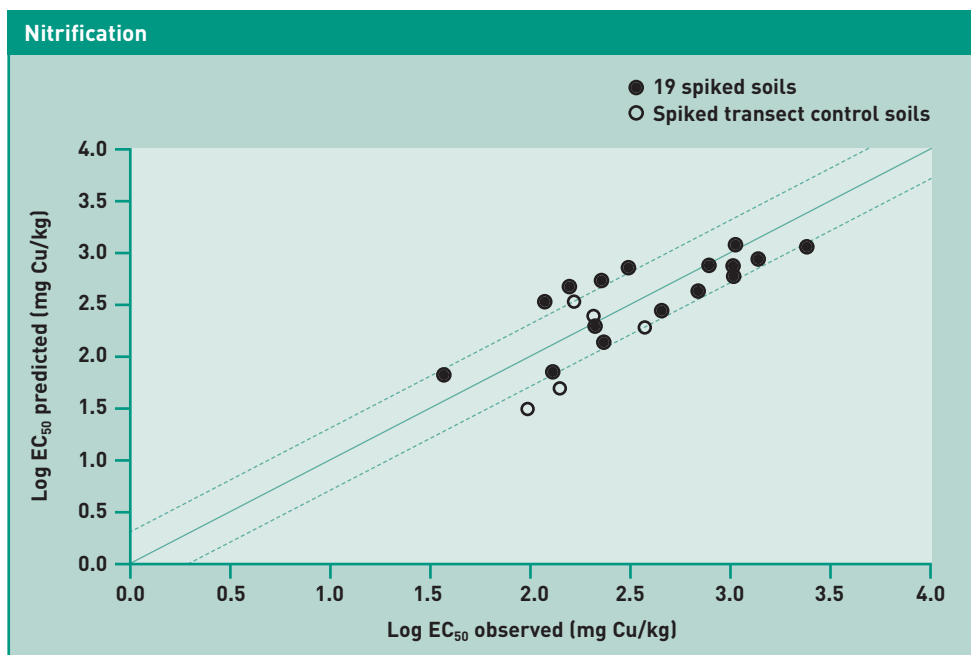
**Table 3: Regressions relating to bioavailability of zinc in soil**

Organism	Statistics	X-variable	Y-variable	Slope [95% Confid. Intervals]	Source
<b>Invertebrates</b>					
<i>F. candida</i>	R <sup>2</sup> = 0.84 Q <sup>2</sup> = 0.78 N=15	Log EC <sub>50</sub> (added zinc in mg/kg)	Log CEC	1.14  (0.84-1.42) p<0.001	Lock et al. (2003)
<i>F. candida</i>	R <sup>2</sup> = 0.89 N=9	Log NOEC	Log CEC	1.19	Lock and Jansen (2001a)
<i>E. fetida</i>	R <sup>2</sup> = 0.77 Q <sup>2</sup> = 0.70 N=14	Log EC <sub>50</sub> (added zinc in mg/kg)	Log CEC	0.79  (0.52-1.06) p<0.001	Lock et al. (2003)
<i>E. fetida</i>	R <sup>2</sup> = 0.80 N=9	Log NOEC	Log CEC	1.18 ± 0.22	Spurgeon and Hopkin (1996)
<b>Plants</b>					
<i>T. aestivum</i>	R <sup>2</sup> = 0.84 Q <sup>2</sup> = 0.74 N=14	Log EC <sub>50</sub> (added zinc in mg/kg)	Log CEC  pH	0.87 (0.45-1.29) p<0.001 0.12 (0.02-0.22) p<0.05	Smolders et al. (2003)
<b>Microbial processes</b>					
PNR	R <sup>2</sup> = 0.55 Q <sup>2</sup> = 0.42 N=13	Log EC <sub>50</sub> (added zinc in mg/kg)	Log background zinc (mg/kg)	0.76 (0.30-1.22) p<0.01	Smolders et al. (2003)
SIR	R <sup>2</sup> = 0.42 Q <sup>2</sup> = 0.13 N=14	Log EC <sub>50</sub> (added zinc in mg/kg)	Log background zinc (mg/kg)	0.76 (0.19-1.33) p<0.05	Smolders et al. (2003)

**Figure 12:** Predicted and observed  $EC_{50}$  (added Zn, mg/kg) for nitrification in spiked soils



**Figure 13:** Predicted versus measured  $EC_{50}$  values for microbial assays with copper (Smolders and Oorts, 2004)





### Empirical models (linear/multiple regression)

Since the conventional BLM-approach is not feasible at the moment for predicting metal toxicity in soils, one has to turn to the application of more empirical models – if validated thoroughly. When sufficient data are available for an organism or a plant, it is possible to determine the soil properties that explain most of the variation in the toxicity thresholds by means of correlations and simple linear regressions between toxicity thresholds and the soil properties. Multiple regressions are calculated by a stepwise procedure that identifies the parameters that explain most of the variation in the dependent variable (i.e., EC<sub>50</sub>, NOEC). The soil parameters that are commonly taken into account are pH, metal background concentration, amorphous iron, aluminium and manganese, total C, organic C, total N, CEC, sand, silt and clay. Similarly, it can be investigated whether soil solution properties (pH, Ca, DOC) can explain the variation in toxicity thresholds based on metal concentration in the soil solution.

Lock and Janssen (2001) found for zinc that acute and chronic toxicity to the earthworm *Eisenia fetida* and the potworm *Enchytraeus albidus* were mainly controlled by pH and cation exchange capacity, the latter reflecting the clay and organic matter content in the soil. Some of the regression models predicting Zn toxicity towards plants and terrestrial organisms are presented in Table 3 (regressions developed by Smolders et al. (2003) and Lock et al. (2003)).

For plants, the EC<sub>50</sub> was significantly related to the CEC and pH, whereas the CEC (measured at soil pH) was the parameter that significantly determined the NOEC for invertebrates. The effect of Zn on soil microbial functions was mainly determined by the Zn-background concentration in the soil sample.

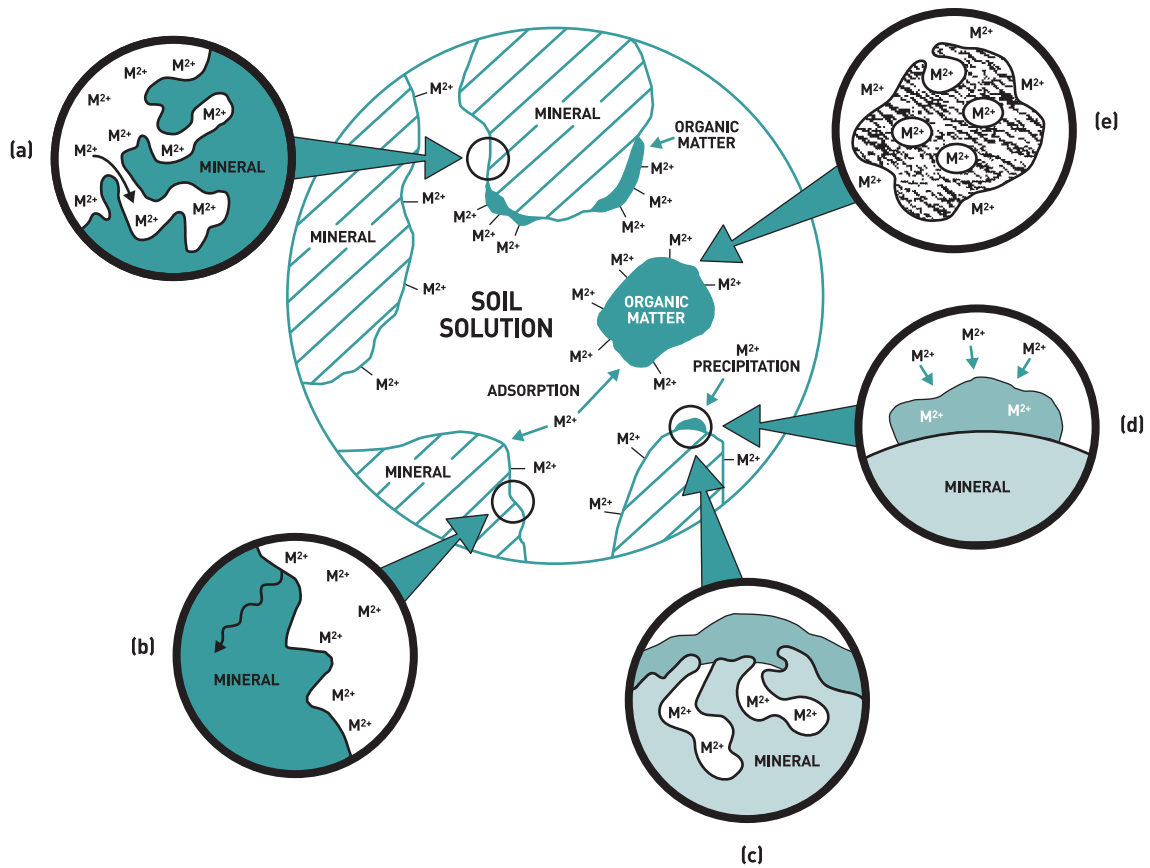
Normalization of soil toxicity data towards a reference soil can be performed with the data reported in Table 3 according to Eq-4:

$$\frac{X \text{ variable}}{X \text{ variable}_{ref.}} = \left[ \frac{Y \text{ variable}}{X \text{ variable}_{ref.}} \right]^{slope} \quad \text{(Eq. 4)}$$

The predicting capacity of these regression models is illustrated in Figures 12 and 13, where the observed effect of Zn and Cu, respectively, on the nitrification is compared with the predicted effect concentration. In both cases predictions did not differ by more than a factor of 2 from the observed EC<sub>50</sub>, whereas the variation of the EC<sub>50</sub>'s determined in different soils was up to two orders of magnitude. This finding indicates that the observed variation among toxicity tests for the same species can be reduced when data are normalized to a reference soil using the developed bioavailability regression models.

These types of models have been developed for copper and zinc and are currently under development for other metals (e.g., nickel, cobalt). The validation exercises with currently available models confirm that these types of multiple regression relationships are useful tools that link bioavailability, soil chemistry and toxicity to soil biota.

**Figure 14:** Adsorption, precipitation and ageing processes in soil. Adsorption and precipitation moves metals from the solution to the solid phase (soil surfaces). Ageing moves metal from soil surface to deeper/stronger in the solid phase through (a) surface pore diffusion, (b) solid state diffusion, (c) occlusion of metals through precipitation of other phases, (d) precipitation of new metal solid phases and (e) occlusion in organic matter (Adapted from McLaughlin, 2001)





#### 4.3.4 Soil metal toxicity under field conditions – consequences for lab-to-field extrapolation

With regard to the bioavailability of metals associated with the solid phase, it has been found that time of contact between soil and metal is a critical factor in determining the bioavailability of essential and non-essential metals. Smolders et al. (2003) and Lock et al. (2003) demonstrated that the adverse effect of elevated zinc toxicity is generally stronger in (freshly) spiked soils than in field-contaminated soils ('leached and aged') at the same total zinc concentrations, due to higher Zn-solution concentrations in the spiked soils. At equal toxicity in the laboratory and the field, a field/lab spike factor ('lab-to-field factor'; L/F-F) can be calculated as:

$$L/F-F = \frac{\text{field Me dose } X_1 \text{ needed in soil } Y \text{ to give toxicity effect } Z}{\text{lab spike Me dose } X_2 \text{ needed in soil } Y \text{ to give toxicity effect } Z}$$

This factor converts the PNEC in the field to the dose in a freshly spiked laboratory soil that yields an equivalent availability (i.e., same pore water concentration) and toxic effect (Smolders and Degryse, 2003). The 'lab-to-field factor' can also be estimated using measured or calculated metal concentrations in laboratory-spiked and field-contaminated soils.

The F/S factor derived for Zn for different scenarios ranged between 1.5 and 15.1. Reported lab-to-field factors for Pb varied between >1.1 and >43 and could not be quantified exactly due to the lack of any toxic signal in the field-contaminated soil (Waegeneers et al., 2004).

As a result, the observed metal toxicity in field-contaminated soils can be reduced to less than 10% of the toxicity found in laboratory-spiked soils. This phenomenon is referred to as attenuation, ageing, fixation, reversion, irreversible binding, adsorption/desorption hysteresis, etc. Evidence of ageing processes is provided by studies of metal extractability and lability (Hamon et al., 1998). The different processes that may explain the lower toxicity observed in the field are depicted in Figure 14 (McLaughlin, 2001). Initial adsorption processes are due to an electrostatic bond between the metal and the charged soil surfaces. As a result, cationic metals are sorbed most strongly at high pH, whereas most sorption of anions occurs at lower pH-levels. In time, the strength of bond may increase due to a rearrangement of the metal on

the surface of the solid phase (diffusion into micropores on the organic or inorganic surfaces) (Figure 14a) or due to a slow diffusion away from the surface into the crystalline structure of the soil mineral (Figure 14b) (Barrow, 1987).

A metal ion can also become unavailable when it becomes occluded due to (co)precipitation, (co)floculation or the formation of another, newly formed (non-metallic) solid phase (Figure 14c). This phenomenon can be observed in seasonally waterlogged soils, where there is a continuous reduction and oxidation process that causes iron and manganese oxides to be solubilized and re-precipitated several times, thus 'burying' available metal within solid forms.

Beside adsorption or occlusion into the mineral phase of the soil, a metal can also precipitate from the soil solution in association with a corresponding anion that is also present in this solution (Figure 14d). This process is most important when metal loadings are high or when the counter ion concentrations are high (e.g., carbonates, phosphates). An example is the precipitation of Pb as hydroxy- or chloropyromorphite in the presence of dissolved phosphate. As these compounds are highly insoluble, added Pb would be largely fixed effectively in the soil.

Similarly to what is observed for the mineral phase, a metal can also be occluded in the structure of an organic molecule that is formed in the soil. A more tight binding of a metal into an organic molecule can also be promoted by diffusion into the molecule (Figure 14e).

All these metal-solid processes lead to a decreased availability of the metal ion in time and thus affect both the beneficial use of a metal (in agricultural context) and the assessment of risk posed by elevated metal concentrations in the terrestrial compartment. Adsorption processes will have a significant effect in reducing soil solution metal concentrations for short contact times of a metal with soils (minutes to hours), whereas it is expected that processes of diffusion, precipitation and occlusion will reduce metal availability and toxicity over a longer period of time. As a result, the observed toxicity in spiked soils with short equilibration times will tend to overestimate metal toxicity compared with field conditions where metals have been in contact with the soil for longer periods, especially for organisms where the soil pore water is the main exposure route.

Unfortunately, due to the few studies in this area, it is currently not possible to draw firm conclusions regarding ageing for each individual metal. Experimental work by Barrow (1986) and Barrow et al. (1989) has indicated that the rate of the 'ageing' reaction is metal-specific, with the rates of ageing as measured by adsorption-desorption hysteresis differing as follows:  
 $Cd < Mo < Zn < Ni$ .

As the actual mechanisms that play a role in metal-soil interactions are not fully understood yet, it remains impossible to state that these toxicity-reducing processes are either irreversible or totally reversible under natural conditions. Acidification and oxidation may lead to a release of adsorbed and precipitated metals (e.g., oxidation of insoluble metal sulfides, oxidation of organic molecules). The reversibility of metal diffusion into micropores or the crystal structure of minerals is situation-dependent. Since diffusion is a process that is driven by concentration gradients (and temperature), the direction of movement is predominantly from available to non-available when there is a continuous input of metal to the soil. In metal-deficient soils, however, or in soils where metals are depleted due to continued uptake by plants or leaching reactions without net additions, a metal mobilization is expected, although at a slower rate than the initial ageing reaction (McLaughlin, 2001).





METALS ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

# BIOCONCENTRATION, BIOACCUMULATION AND BIOMAGNIFICATION OF METALS

# 05



## 05. Bioconcentration, bioaccumulation and biomagnification of metals

Bioconcentration<sup>2</sup>, bioaccumulation<sup>3</sup> and biomagnification<sup>4</sup> are important for a variety of reasons: bioaccumulation is one of the criteria frequently used in hazard identification and risk assessment (e.g., along with persistence and toxicity – PBTs); bioconcentration and bioaccumulation factors are proposed for use in evaluating bioaccumulation; bioaccumulation infers a propensity to biomagnify and impart increased toxicity to organisms at higher trophic levels in the food-chain; and bioaccumulation of essential elements is a mechanism to regulate the necessary concentration of an element to the organism. Of the metals so far investigated, methylmercury and possibly selenium are known to biomagnify in aquatic organisms (Parametrix, 1995) and cadmium, methylmercury and possibly lead are the only metals that may have the potential to biomagnify in terrestrial organisms (Beyer, 1986; Eisler, 1987; Parametrix, 1995). In hazard classification, bioconcentration factor (BCF) and bioaccumulation factor (BAF) thresholds above which a substance is deemed bioaccumulative are generally set at 500 to 5000 for all substances, including metals. For highly toxic synthetic compounds, which are persistent and have the ability to biomagnify, the use of bioaccumulation may well provide very useful information. However, for naturally occurring substances such as metals, bioaccumulation is complex and many processes are available to modulate both accumulation and potential toxic impact. These mechanisms are evidenced by adaptation and acclimation. All biota will naturally accumulate metals at least to some degree without deleterious effect. Furthermore, a number of metals are essential elements, and thus bioaccumulation in itself does not indicate the potential for adverse effects (McGreer et al., 2002).

Metal bioaccumulation is an important process in the homeostatic regulation whereby organisms obtain metals essential for biological function, such as enzymatic and metabolic reactions

<sup>2</sup> Bioconcentration is the increase in concentration of a substance in organisms (or specified tissues thereof) relative to the concentration of the substance in the environmental medium (generally water) to which it is exposed. The bioconcentration factor is a unitless value calculated by dividing the steady state tissue concentration of a substance by the steady state environmental concentration, normally water.

<sup>3</sup> Bioaccumulation is the amount of a substance taken up by an organism from water (bioconcentration) as well as ingestion via the diet and inhalation. The bioaccumulation factor is a unitless value calculated by dividing the steady state tissue concentration by the steady state environmental concentration, assuming uptake via both water, food and air.

<sup>4</sup> Biomagnification is the process by which the tissue concentration of a bioaccumulated substance increases as it passes up the food chain through at least two levels (Parametrix, 1995).

(Adams et al., 2000). It should also be noted that non-essential metals are often regulated to varying degrees because the mechanisms for regulating essential metals are not metal-specific. Bioaccumulation is defined as the uptake and net accumulation of a chemical substance by an organism from its environment and/or diet.

In the case of aquatic organisms, the ratio of the tissue concentration to the water concentration is termed the bioaccumulation factor. BAF estimates assumed exposure from water or diet (including solid phases such as organic and inorganic particles loaded with metals that may be released inside the body) or both and are often derived from field data. For plants, BAFs also include metals aerially deposited on leaves as well as those in soil particles adhering to roots. BAFs for terrestrial plants do not take into account this fraction of metals, as these BAFs are frequently determined in hydroponic culture. In oligochaetes, BAFs also include metals adhering to the animal's body surface. BAFs can thus be defined as the ratio of the metal concentration in an organism to that in the surrounding medium at steady state.

Bioconcentration factors are used to define the ratio of the tissue or whole body concentration to the water concentration at steady state and assume water exposure and no dietary exposure. BCFs are typically derived in laboratory experiments. Strictly speaking, metal bioconcentration in sediment and soil systems is the net accumulation of a metal in or on an organism from pore water only. BCFs are often used instead of BAFs because the latter are frequently not available. Since bioconcentration is usually defined as the uptake of a substance from the surrounding aquatic environment, it is not a relevant parameter to describe the uptake of metals by terrestrial organisms. Therefore, bioaccumulation and possibly biomagnification are more relevant parameters for describing metal uptake by terrestrial organisms. For terrestrial organisms, BAFs are generally low (Parametrix, 1995). Most metals can be absorbed through the gut in invertebrates, reptiles, birds and mammals. The gut wall is composed of a gut epithelium, muscle layer and chloragocytes (Hopkin, 1989; Parametrix, 1995). However, uptake can be controlled where both non-essential and essential metals in excess of metabolic requirements are sequestered by the chloragocytes and subsequently excreted back into the lumen of the gut. In general, metal bioaccumulation does occur

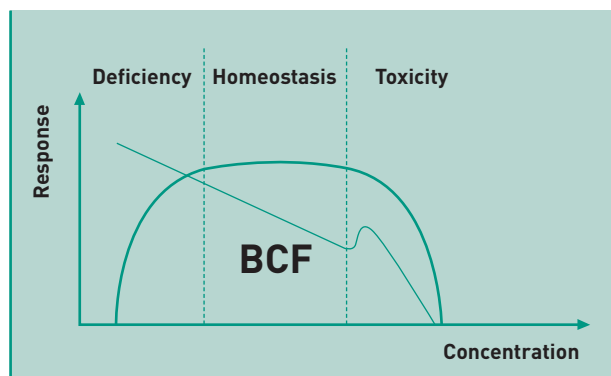


in terrestrial food-chains, but bioaccumulation is mostly low.

Aquatic biota regulate their internal concentrations of essential metals by active regulation, storage or a combination of both. As a result of these processes – and more specifically due to active regulation – an inverse relationship exists between metal water concentrations and the corresponding BCF. Thus, at low water concentrations, organisms are accumulating essential metals (and often non-essential metals via the same uptake mechanisms) to meet their metabolic requirements. At higher water concentrations, organisms with active regulation mechanisms are able to excrete excess metals or limit their uptake. This hypothesis applies to most organisms for both essential and non-essential metals (Brix and Deforest, 2000). As a result, metal concentrations in tissue based on a range of exposure concentrations may be quite similar, but the BCFs will be quite variable (i.e., higher BCFs at lower exposure concentrations and lower BCFs at higher exposure concentrations). When toxicity is elicited, a linear decreasing BCF for metals is generally not observed. One usually finds first an increase in BCF and then a rapid decrease in BCF, since organisms that are in the process of dying do not take up any further metals or cannot activate further bounding mechanisms. Often there is thus a peak in the BCF or a minimum plateau at the onset of toxicity. Furthermore, BCFs determined at the same exposure concentration in different experiments can be highly variable (McGreer et al., 2002). Consequently, an individual BCF provides little information on the bioaccumulation potential of a metal. Moreover, it is possible that BCFs increase again once a critical concentration in the surrounding medium is exceeded. The mechanism of metal regulation and varying BCFs is shown in Figure 15.

Most metals bioaccumulate to some degree in the tissues of aquatic organisms; however, the extent of bioaccumulation varies, depending on the bioavailability of the metal and the specific organisms being considered, as well as exposure concentrations and environmental factors (Memmert, 1987; Parametrix, 1995). Some species accumulate metals to high levels (e.g., zooplankton), while other species such as fish closely regulate internal concentrations or sequester the metal with cellular binding proteins (e.g., metallothioneins) (Hodson, 1988; Rainbow and White, 1990; Parametrix, 1995). For aquatic organisms, bioconcentration is the most important route of

**Figure 15: Metal regulation showing ranges of deficiency, homeostasis, toxicity and bioconcentration factor. The BCF is negatively correlated with the environmental concentration.**



uptake for most metals. Only for a limited number of metals (mercury, selenium) has it been suggested that the food component is more important than the water component in metal bioaccumulation (Biddinger and Gloss, 1984; Dallinger et al., 1987; Sandholm et al., 1973; Tuner and Swick, 1983; Parametrix, 1995).

BCFs derived from field data should be used with caution, as they do not necessarily reflect steady state. As a result, exposures may be underestimated for BCF values, resulting in an elevated BCF value. In addition, the BCF alone gives only a partial indication of the potential for a substance to accumulate. Additional data on the bioavailability, uptake and depuration kinetics, metabolism, organ-specific accumulation and the level of bound residues are also required. For example, differences in gut physiology, renal excretion rates and egg production influence the toxicokinetics of the metal. These data are often not readily available, and the BCF must be estimated using physico-chemical and structural properties of a substance, leading to increased uncertainty. The ability of some species to more rapidly produce protective proteins such as metallothionein after exposure to metals is a toxicodynamic feature leading to interspecies extrapolation uncertainty. In cases where some species are able to regulate or store metal residues in their tissues without experiencing toxic effects, extrapolations between species used to assess bioaccumulation and toxicity can be especially problematic. Furthermore, in the context of essential elements, the required concentration of an EE must be taken into account. As mentioned, accumulation of some essential metals may be

regulated by some organisms to maintain necessary concentrations in cells. Although for some species there is a relationship between BCFs and chronic toxicity (e.g., *Hyalella azteca*; Borgmann et al., 1996), BCFs should in general not be used either as a predictor of adverse effects or alone to establish criteria for the bioaccumulation or biomagnification potential of metals. Dietary requirements should be taken into account as well in the evaluation of the bioaccumulation and bioconcentration of EEs. BCFs for essential metals could be calculated or a deficiency-toxicity model could be developed (Parametrix, 1995). BAFs should be expressed as the ratio of metal in the organisms to the bioavailable fraction medium under consideration.

The food source is an important factor in determining the effect of metals in the food chain. Laboratory studies sometimes fail to mimic the effects of dietborne metals on fish in natural environments. Effects in the field are sometimes encountered at much lower concentrations than in laboratory studies with (freshly) spiked food (often short time exposure of the food to high concentrations of metals). This might well be due to a combination of the quality of the food (e.g., field invertebrates affected by metals are less nutritious) and of metals incorporated in the food source being more bioavailable than metals added to the food in lab studies (Clearwater et al., 2002; Woodward et al., 1994; Paripatananont and Lovell, 1995, 1997). Therefore, it is important that laboratory studies investigating the effects and uptake of dietborne metals use diets that mimic as closely as possible those in the natural environment (Clearwater et al., 2002). The bioavailability of metals in food is not fully understood. Sometimes low availabilities are encountered – for instance, when the metals in the prey animals are stored in granules.

Apart from the 'normal' food sources, animals can also accidentally or deliberately ingest sediments. Fish, for instance, can be exposed to metals in the sediment as well as in food. Incidental ingestion of sediments increases dietborne exposure of fish to metals. Many fish tend not only to consume prey items such as worms and shellfish, but also to deliberately ingest sediments as a feeding strategy or to incidentally ingest significant quantities of sediment that are present in the digestive tract of the invertebrates, attached to the surface of the invertebrates or in the water when the invertebrate is sucked into the fish's mouth. Since sediments

tend to accumulate high concentrations of metals, the incidental ingestion of sediment might constitute a significant dietborne metal exposure for some species (Clearwater, 2002). However, the bioavailability of metals in ingested sediment (and even in food in general) is not very well understood. This applies not only to fish but also to other animals, such as birds and even mammals.

Information on toxicity of metals to wildlife under field conditions is limited, and in many cases the metal form used in laboratory studies is not the dominant form in the field. A solution for the problem of extrapolating results may – for some metals – be the use of critical tissue residues. Most lead field studies, for instance, have been conducted with pure elemental lead, while most laboratory studies have been performed with lead acetate. This makes extrapolation from lab to field dietary exposure problematic. The best approach in this case could be the use of critical tissue residues, because liver lead levels indicative of lead poisoning are well established (Beyer et al., 1996). The form of metals in tissues, however, may influence availability and effects.

Unless metal concentrations in pore water serve as the denominator for the ratio, soil and sediment BAFs are usually termed biota-soil or biota-sediment accumulation factors (BSAFs) and usually expressed on a total metal- and weight-adjusted whole organism basis. For sediments it is important that the distribution of metal between pore water and sediment phases reflects what occurs in nature. Quite often, insufficient time is allowed for equilibrium to occur, and as a consequence the dissolved exposure pathway is overestimated, which may yield overestimates of true BSAFs. Further, the bioavailability of metals in sediments can be described well by the SEM/AVS concept.



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## 06. References

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