Texte

Texte
04
09
ISSN
1862-4804

Incorporation of Metal Bioavailability into Regulatory Frameworks

Umwelt
Bundes
Amt
Für Mensch und Umwelt

UMWELTFORSCHUNGSPLAN DES
BUNDESMINISTERIUMS FÜR UMWELT,
NATURSCHUTZ UND REAKTORSICHERHEIT

Forschungsbericht 360 12 018
UBA-FB 001249

Texte
04
09

ISSN
1862-4804

Incorporation of Metal Bioavailability into Regulatory Frameworks

von

Wolfgang Ahlf
Susanne Heise

Technische Universität Hamburg-Harburg
Institut für Umwelttechnik und Energiewirtschaft

Im Auftrag des Umweltbundesamtes

Herausgeber: Umweltbundesamt
Postfach 14 06
06813 Dessau-Roßlau
Tel.: 0340/2103-0
Telefax: 0340/2103 2285
Internet: http://www.umweltbundesamt.de

Redaktion: Fachgebiet IV 2.3
Wiebke Drost

Dessau-Roßlau, März 2009

Berichts-Kennblatt

1. Berichtsnummer
UBA FB 001249

2.

3.

4. Titel des Berichts:
Berücksichtigung aktueller Konzepte zur Bioverfügbarkeit in der Umweltrisikobewertung von Metallen

5. Autor(en), Name(n), Vorname(n)
Ahlf, Wolfgang
Heise, Susanne

8. Abschlussdatum
1 1. August 2008

9. Veröffentlichungsdatum
März 2009

6. Durchführende Institution (Name, Anschrift)
TuTech Innovation GmbH
Harburger Schloßstraße 6-12
21079 Hamburg

10. UFOPLAN-Nr.
FKZ 36012018

11. Seitenzahl: 47

7. Fördernde Institution (Name, Anschrift)
Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau

12. Anz. Literaturangaben: 93

13. Anz. Tabellen: 3

14. Anz. Abbildungen: 6

15. Bemerkungen

16.Kurzfassung

Inwiefern Metalle für Organismen verfügbar sind, hängt unter anderem von den geochemischen Gegebenheiten ab, welche die Metallspeziation bestimmen. Das Bioligandenmodell (BLM) und das Konzept von sulfid-gebundenen und somit nicht verfügbaren Metallen(SEM/AVS) wurden entwickelt, um die Bioverfügbarkeit von Metallen abzuschätzen. Basierend auf einer Literaturstudie mit besonderem Augenmerk auf unterschiedliche Herangehensweisen, diskutiert dieses Gutachten die Einbeziehung der Bioverfügbarkeit von Metallen in der Risikoabschätzung im Kontext der Altstoffbewertung und Bestimmung von Umweltqualitätszielen. Hierzu wurden die vom Internationalen Metallverband(ICMM) verfasste Anleitung zur Risikoabschätzung von Metallen (MERAG) und die Wasserrahmenrichtlinie herangezogen. Das Gutachten geht der Frage nach, was die Hauptaufnahmewege von Metallen in den Kompartimenten Wasser, Sediment und Boden sind, ob diese Aufnahmepfade durch die vorgeschlagenen Modelle und Konzepte hinreichend abgedeckt werden und was zu berücksichtigen ist, wenn die Bioverfügbarkeit von Metallen in der Regulation einbezogen wird.

Das Gutachten zieht den Schluss, dass Metallkonzentrationen durch adäquate Bioverfügbarkeitsfaktoren berücksichtigt werden sollten, dass aber die vorgeschlagenen Modelle und Konzepte BLM und SEM/AVS dies nur unzureichend abdecken, da hier angenommen wird, dass die freie Ionenkonzentration die wichtigste ist. Neben dem geochemischen Zustand, welcher die freie Ionenkonzentration bestimmt, fallen jedoch ebenfalls partikulär oder labil gebundene und komplexierte Metallionen ins Gewicht. Außerdem ist das Verhalten des jeweiligen Organismus in der Umwelt, z.B. durch das Fressverhalten und die Bioturbation im Sediment bei der Betrachtung der Bioverfügbarkeit relevant. Die Berücksichtigung der Bioverfügbarkeit sollte deshalb durch diese Optionen erweitert werden. Das Gutachten schlägt als Alternative eine Erweiterung des DYNBAM vor, welches als ein biodynamisches Modell zusätzlich zu frei gelösten Metallionen auch die Aufnahme von Metallen über die Nahrung mit berücksichtigt. Das vorgeschlagene erweiterte konzeptionelle Modell bezieht bei der Bioverfügbarkeit auch labil gebundene und komplexierte Metalle mit in die Betrachtung ein. Anhand von Fallbeispielen erläutert das Gutachten, wie wichtig bei der Herleitung von Umweltqualitätszielen im Rahmen der Wasserrahmenrichtlinie die Einbeziehung der Sedimente ist; dass allgemeine Umweltqualitätsziele unsicher sind und daher das Metallo-Region Konzept bevorzugt werden sollte.

17. Schlagwörter: Metalle, Biologische Verfügbarkeit, BLM, AVS/SEM, DYNBAM

18. Preis

19.

20.

Report Cover Sheet

1. Report No.
UBA FB 001249

2

3

4. Report Title: Incorporation of Metal Bioavailability into Regulatory Frameworks

5. Author(s), Name(s), First Name(s)
Ahlf, Wolfgang
Heise, Susanne

8. Report Date
August 2008

9. Publication Date
March 2009

6. Performing Organisation (Name, Address)
TuTech Innovation GmbH
Harburger Schloßstraße 6-12
21079 Hamburg

10. UFOPLAN-No.
FKZ 36012018

11. No. of Pages: 47

7. Sponsoring Agency (Name, Address)
Umweltbundesamt [German Environment Agency]
Wörlitzer Platz 1
06844 Dessau

12. No. of References: 93

13. No. of Tables, Diagrams: 3

14. No. of Figures: 6

15. Remarks

16. Abstract:

The degree to which metals are available to organisms varies depending in part on the site-specific geochemical conditions controlling metal speciation. The Biotic Ligand Model(BLM) and the concept of sulphide bound metals described by the ratio of Simultaneously Extracted Metals and Acid volatile sulphide concept(SEM/AVS) have been developed to consider the bioavailability of metals. On the basis of a review on the literature relating to bioavailability approaches, this work discusses the incorporation of metal bioavailability into the risk assessment of metals in the context of the Existing Substances Regulation and into guidelines such as the guidelines for establishing Ambient Quality Criteria. Focus is put onto the metal risk assessment guidance documents for environment and health, MERAG (2007), published by the International Council on Mining and Metals and the Water Framework Directive. This report addresses current knowledge of bioavailability and reviews experimental evidence of the main exposure routes of metals for the compartments water, sediment, and soil. The question whether the suggested approaches are suited to assess the bioavailability of metals in the different compartments and what has to be considered in order to incorporate bioavailability of metals into regulatory frameworks is being addressed.

The report concludes that metal concentrations should be adjusted by appropriate bioavailability factors but that the current suggested approaches BLM and SEM/AVS are insufficient. Both approaches assume that the free ion concentration is the most relevant exposure pathway. However apart from geochemical conditions, which control free metal concentration, bioavailability is additionally a result of contaminant/particle interaction and can be superimposed by organisms' activity, like feeding or burrowing behaviour. The report proposes that the influence of labile bound metals and metal complexes should be implemented in refined models and to extend the scope of bioavailability. The report suggests a synopsis with basic model DYNBAM which is a dynamic multi-pathway bioaccumulation model and also considers the uptake of metals via food. As an extension of DYNBAM the authors suggest a conceptional model which also considers, labile bound and complexed metals as an additional source. Concerning the Water Frame Directive the report states that generalized Environmental Quality Standard (EQS) would face a high uncertainty and shown on case studies that ignoring sediment in the risk assessment schemes could lead to major risks. Hence, sediments should be included in EQS derivation, the metalloregion concept should be applied and metal-specific biodynamics should be considered using DYNBAM.

17. Keywords: metals, bioavailability, BLM, SEM/AVS, DYNBAM, particle associated metals

18. Price

19

20

Contents

1 Introduction

Metals discharged into aquatic systems are mostly adsorbed on suspended particles and finally accumulate in sediments. Since the toxicity of metals have been recognized as important environmental hazard, extensive research was conducted on the dose-effect relationship under different geochemical conditions. In those studies during the 1970s and 1980s, exposure was focused on waterborne metals and factors affecting metal bioavailability. Especially the influence of water composition on toxicity such as hardness and pH was discovered. Regulatory authorities developed Water Quality Criterias, which based on a compilation of measurements and on mechanistic statistical relationships.

During the past two decades numerous studies have shown that the degree to which metals are available to aquatic and terrestrial organisms varies depending on the site-specific geochemical conditions controlling metal speciation. For instance, sediment-water interactions in natural aquatic systems play an important role in controlling transport and exposure processes of metals. At the same time the mechanistic understanding of metals toxicity developed, whereas the significance of free metal ions were accepted. Consequently, this field of research has recently expanded, and we have advanced models linking knowledge of physiological mechanisms to the many factors that alter metal toxicity in nature. This mechanistic understanding has been incorporated into the risk assessment of metals in the context of the Existing Substances Regulation. At present, proposals have been made to incorporate this mechanistic understanding of exposure and toxicity into regulatory frameworks. Thus, it is time to review new informations of the last years and discuss the potential incorporation of metal bioavailability into guidelines such as the guidelines for establishing Ambient Quality Criteria.

1.1 Goals and Objectives

This work was initiated by the Umweltbundesamt (UBA) and has been focused on reviewing the metal risk assessment guidance documents for environment and health, MERAG (2007). The Metals Environmental Risk Assessment Guidance (MERAG) was published by the International Council on Mining and Metals. The concepts are presented in the series of independently reviewed MERAG ‘fact sheets’, which were the outcome of discussions between stakeholder, scientific and regulatory experts (for further details visit www. metalsriskassessment.org).

The goal of this report is to critically review the literature of the last years relating to bioavailability approaches. The current information about exposure and risk assessment of metals in the compartments water, sediment, and soil are summarized and discussed in order to evaluate the scientific basis of the MERAG concept.

Our specific objectives are as follows:

- Extensively review published and gray literature related to metals with the keywords: Accessibility, Bioavailability, Regulatory Incorporation, and Risk Assessment.
- Critically review the theoretical basis by which environmental parameters affect the bioavailability and toxicity of metals.

- Critically review experimental and field evidence that supports or does not support proposed mechanisms of bioavailability, including the possible use of geochemical speciation programs and BLM approach for exposure and risk assessment (e.g. SSDs) for the compartments water, sediment, and soil.
- Identify additional research needed to improve the mechanistic basis for deriving quality criteria for metals, and evaluating effects in local and regional monitoring programs.

1.2 Background and Overview

Considerable effort is being expended to incorporate bioavailabiliy concepts into regulatory frameworks for metals. The background is, that it is widely accepted that the degree to which metals are available to aquatic and terrestrial organisms varies depending on the site-specific geochemical conditions controlling metal speciation. The MERAG fact sheet No. 5 (2007) presented a bioavailability concept for refining the risk assessments by taking into account bioavailability in the compartments water, sediment, and soil. Depending on the compartment under investigation different levels of bioavailability refinement can be distinguished (Figure 1.1), which will be briefly explained step by step. However, at first a statement from the MERAG publication has to be emphasised. It was clearly assumed that in the environment organisms are not only exposed through water but also via the dietary route. Nevertheless, the science on the incorporation of the dietary route was seen as very preliminary and as such more or less not considered. However, concurrent exposure to waterborene and dietborne metals generally provides more realism than exposure to metal uptake through only one route. To our opinion the choice between waterborne and dietborne metal exposures is acceptable for a reliable risk assessment only, if it is clear that one exposure route superimposes the other one. We think, the exclusion of dietborne metals from the start decimates the applicability of the concept already at the beginning.

In general, from the perspective of a risk assessment or in Environmental Quality Standard (EQS) setting, it is crucial to understand which geochemical characteristics influence the toxicity of metals and metal compounds. In order to reduce the uncertainty and to increase the ecological relevance of the assessment both effects and/or exposure data should be normalized to the bioavailable metal fraction. Depending on the knowledge level for the metal/metal compound under investigation, bioavailability can be introduced at different levels according to the tiered approach in Figure 1.1. The most simple form makes use of the information on partitioning and the amount of potential bindig sites on solids present in the aquatic compartment to translate the total metal concentration into a dissolved concentration (step 1). For the terrestrial compartment the dissolved fraction can be assessed through the use of empirical relationships of toxicity as a function of soil physical and chemical properties such as pH, cationic exchange capacity, and organic matter. For metals compounds that exhibit a strong preference to bind with sulfides the SEM-AVS concept could be useful to quantify the amount of metals not bound to the sulfide pool which provides an estimate of potentially bioavailable metals in anoxic sediments.

A further refinement of this approach would be to estimate the true free ionic metal fraction by using metal speciation programs (e.g. WHAM, MINTEQ etc.) taking into account the presence of important binding ligands and competing cations (step 2). In step 3, for some metals toxicity based models are available or under development that range from simple regression models to the more refined Biotic Ligand Models (BLM) which allows a mechanistic understanding of metal toxicity. The BLM has been proposed for use in European Union (EU) risk assessments, assuming that the biotic ligands are the target sites for toxic effects (further details in chapter 2). If environmental conditions and geochemical influences are comparable within a region, a bioavailability assessment model could be allocated to a region. Selection of methods and approaches for incorporating bioavailability corrections will depend on the aim and scope of the assessment, e.g. for generic chemical safety programs (e.g. the EU Existing Substance Program) or local environmental impact studies (EQS setting). Furthermore, metal specific considerations should always be taken into account (e.g. affinity of the metal to bind with sulfides differs greatly, and separate BLMs must be developed for each metal).

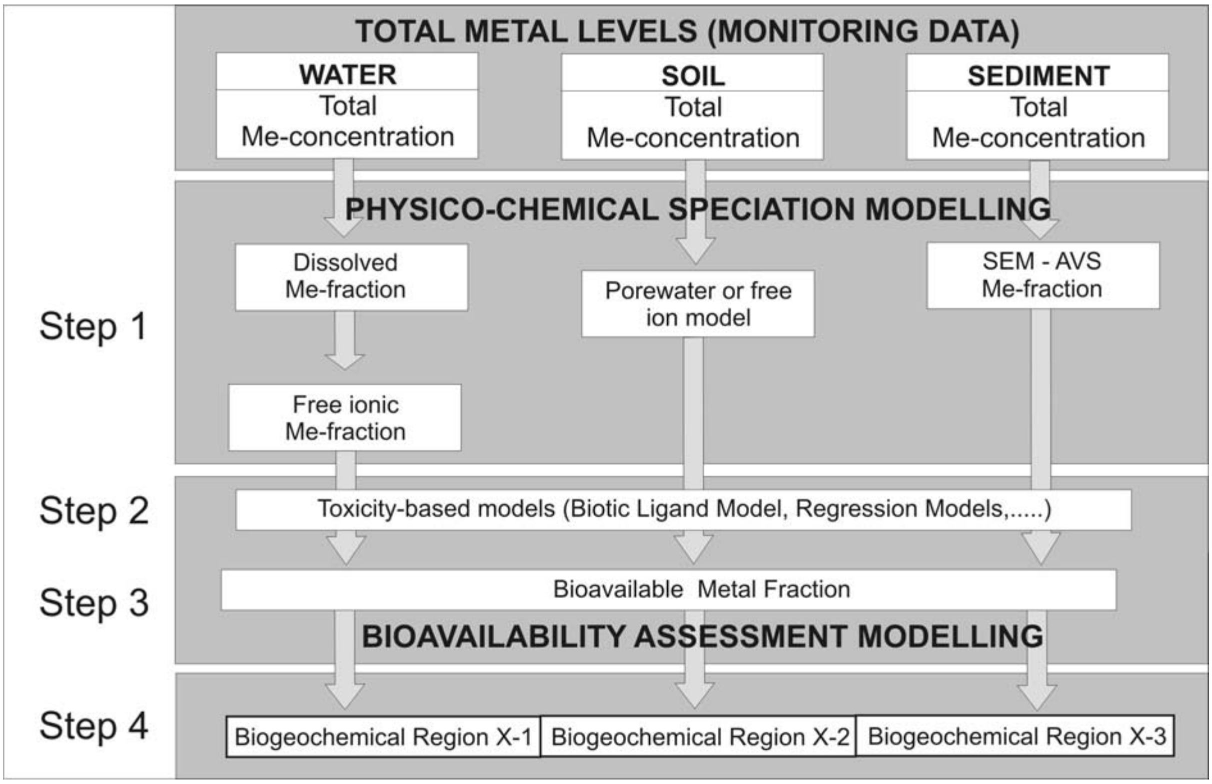


Fig. 1.1: Refinement levels for the incorporation of bioavailability concept for the water, sediment and soil compartment. (MERAG, 2007a; modified)

Depending on the compartment under investigation different levels of bioavailability refinement can be distinguished. The objective of this report is to produce a different view on bioavailability of metals in different environmental compartments. If we do accept the complexity of this process, how can we characterize and predict exposure concentrations? The metal-specific exposure factors discussed here will contribute to the concept of using bioavailable fractions in estimates of PEC and PNEC, which should improve the basis of risk assessments. However, the limitations of the models used (e.g. BLM) must be recognised and any risk assessment for metals should clearly state all assumptions about duration of exposure and what uncertainties are added to the risk model as a consequence.

This report is organized into chapters that address current knowledge to understand metal bioavailability and to implement a mechanistically based concept for regulatory purposes. In Chapter 2, we provide the theoretical basis for the bioavailability of metals to organisms, including explanations of the principles behind the BLM. In Chapter 3, we review experimental evidence of the main exposure routes of metals for the compartments water, sediment, and soil. The different compartments have been evaluated if existing approaches are suited to make obvious the uptake of bioavailable metals. Chapter 4 will state what has to be considered incorporating bioavailability of metals into regulatory frameworks. The closing chapters 5 and 6 are dealing with the environmental risk assessment of metals in relation to the Water Framework Directive.

2 Theoretical Basis for Bioavailability of Metals

2.1 Introduction

When toxicity tests were first conducted with metals, the aim was to find dose-response relationships for different test species. Typically, a pollutant is added to a test system, such as aquariums with daphnia, until a clearly distinguishable effect is observed. At the beginning, in the absence of a bioavailability concept, researchers assumed mortality and sublethal effects were related to the total metal-concentration in the exposure medium. As the discipline has developed, this perception has altered, because the scope of ecotoxicological evaluations has become more sophisticated. Aquatic toxicologists and chemists recognized, that if cationic metals form complexes with inorganic anions and organic matter that the toxicity of the metals to aquatic organisms can decrease. At present, it is generally agreed knowledge that the total contaminant mass in water, soil or sediment is only toxic to test organisms or ecological receptors in parts. In an extreme case, contaminants identified as hazards may not be available to organisms at all. The risk could be minimized making the pollutants not bioavailable, thus eliminating the exposure of organisms to the hazard. Indeed, bioavailability studies are frequently performed to justify site cleanup goals that are more financially or technically feasible and that involve leaving appreciable amounts of contaminant mass in place, while still being protective of public health and the environment (Ehlers and Luthy, 2003). Such pragmatic approaches have not fully elucidated the mechanism why a fraction of a metal concentration is the hazardous one.

2.2 Definition

In a chemical speciation approach, the bioavailable fraction can be defined as one chemical species or the sum of several chemical species in the exposure medium. In a biological approach, the bioavailable fraction can be defined as the portion of the total amount in the exposure medium that correlates with the amount of metal measured as body burden. Since a complete definition of bioavailability does not exist, the term bioavailability has evolved passing from a very simple conception to a complex concept bound to abiotic and biotic aspects.

A consensus exists in the literature with respect to the key processes that define the interaction of trace metals with aquatic organisms. Trace metals are mainly transported into biological cells as metal ions. Therefore the physicochemistry of the metals in solution modifies the concentration of accumulated metal. Now, if researchers establish dose-response-relationships they distinguish between nominal, free, internal, and target concentrations (Escher and Hermens, 2004). Recently, it has become clear that geochemical processes not alone control metal bioavailability, but reactions with different binding sites of the biological surface still play an important role (Worms et al., 2006). This means, the portion of the total amount in the exposure medium that correlates well with the observed toxicity is defined as the bioreactive fraction. Consequently, the bioreactive fraction is always less than or equal to the bioavailable fraction. In addition, toxicity does not depend on total accumulated metal concentration but is related to a threshold concentration of internal metabolically available metal. Body burden metal concentrations are interpreted in terms of different trace metal accumulation patterns, dividing accumulated metals into two components, metabolically available metal and stored detoxified metal. Toxicity ensues when the rate of metal uptake from all sources exceeds the combined rates of detoxification and excretion of the metal concerned (Fig. 2.1).

There is no doubt that a large number of parameters can determine the overall biouptake process and the subsequent biological effects. Bioavailability is a complex result of contaminant/particle interaction and can be superimposed by organisms' activity, like feeding or burrowing behaviour. This means it is unlikely that a simple chemical fractionation method will be developed which imitates an all encompassing bioavailability (Ahlf and Förstner, 2001). Due to this reality, as discussed in Meyer (2002), metal bioavailability may be more of a conceptual term and not a precisely measured parameter.

2.3 Conceptual Models

From the biological point of view, bioavailability of metals can be summarized as the ability to be taken up by and presumably across a biological membrane. Focussing on the interactions between metals and surfaces of organisms, a basic concept was presented by Worms et al. (2006). We briefly summarize the processes, link the concept with the BLM approach, and offer subsequently a synopsis with an extended basic model.

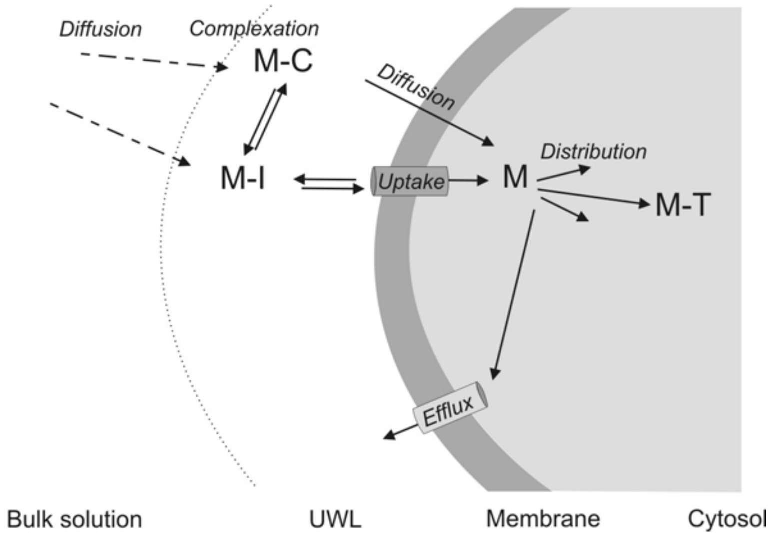


Fig. 2.1 Conceptual model of the main processes and sources for uptake of trace metals at a biological membrane. (M-C: metal complex, M-I. metal ion, M-T: metal at target site, UWL: undisturbed water layer)

According to the concept of Worms and coworkers (2006) a metal ion diffuses as a free ion or as a metal-ligand complex from the bulk solution across a thin undisturbed water layer and reaches the surface of the plasma membrane (Figure 2.1). Although the chemical equilibrium assumption is perhaps unrealistic in dynamic natural media, equilibrium models such as the free ion activity model (FIAM) have been overall very successful in predicting bioavailability (Calace, 2006). This particular mechanism of metal uptake and metal toxicity was related to free metal ion activity only. The metal might also be transported across the plasma membrane to internal sites of either stored up or toxic action after distribution processes. The relationship between metal accumulation and toxicity is influenced by physiological activity and sets the stage for a realistic understanding of the significance of trace metal concentrations in aquatic organisms (Rainbow, 2007).

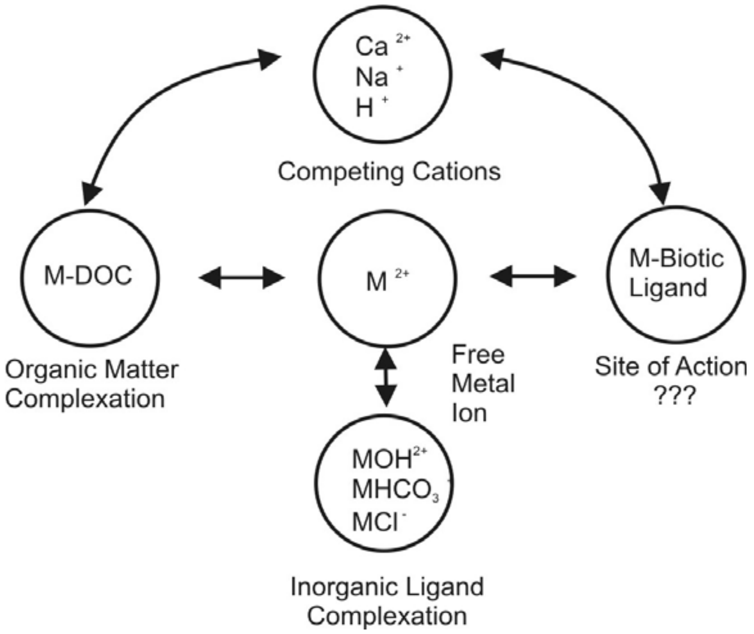


Fig. 2.2 Schematic diagram of the biotic ligand model (from Di Toro et al. 2001; slightly modified)

As a logical extension, a biotic receptor can be included into a geochemical speciation program as an additional ligand if stability constants are specified for its binding with the metal of concern. The geochemical speciation model (e.g. WHAM IV) calculates the amount of free metal ions, which are in equilibrium with the biotic ligand, simultaneously taking into account the modifying effects of the various dissolved ligands and particles in the water column and the presence of other cations that compete with the binding sites on the biotic ligand (Figure 2.2). In sediments the BLM was not practicable because there are other partitioning phases for sediments that must be considered. However, Di Toro et al. (2005) demonstrated that when sediment concentrations, computed by assuming an equilibrium between the critical metal concentration on the biotic ligand (i.e. the test organism) and sediment particulate OC, are compared to critical concentration (BLM) for the sediment test species, toxicity can be predicted for detailed studies.

The biotic ligand model assumes that the free ion metal concentration is responsible for uptake and consequently toxicity might occur after binding to target sites. The overall processes are simplified to a thermodynamic equilibrium, where the complex situation is reduced to a steady state situation. This assumption holds true if diffusion is the only additional delivery process for free ions into the undisturbed water layer. However, if the uptake rate is rapid compared to the rate of diffusion of free metal species to the cell surface the diffusion gradient is steepend. Under these conditions metal complexes may dissociate to counteract the local perturbation to equilibrium. In this situation, metal uptake is controlled by kinetic factors.

The ultimate goal of exposure assessment is to estimate the target dose or biologically effective dose. Measuring the actual dose or toxicant concentration at the target site is usually not feasible, but simple partitioning models and more complex kinetic models offer the possibility of estimating the dose at the target site. As pointed out in detail by Batley et al. (2004) there has been a polarization of research opinions whether metal uptake by organisms is under thermodynamic or kinetic control. The selection of mechanistic explanation is crucial in determining which exposure concentration should be used in toxicity models. For systems where bioavailability is assumed to be determined by thermodynamic factors, measurement or calculation of the equilibrium concentrations of free metal ion is recommended. Labile metal concentrations will be better predictors of metal bioavailability under more dynamic conditions and we think that this is the most common situation in nature.

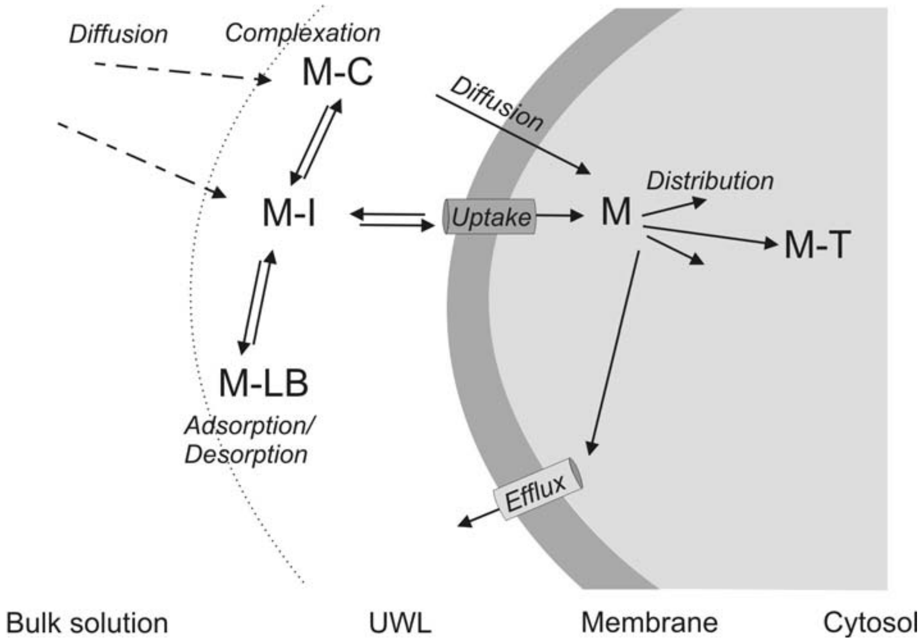


Fig. 2.3 Conceptual model of the main processes and sources for uptake of trace metals at a biological membrane. (M-C: metal complex, M-I. metal ion, M-LB: labile bound metal, M-T: metal at target site, UWL: undisturbed water layer)

On top of it, if contaminated particles are present there is a dramatic change of mass transport into the undisturbed water layer, as it was demonstrated for hydrophobic substances (Oomen et al., 2000). We think, in such cases particle-bound metals also contribute to biouptake according to advective solute supply. Labile bound metals could be desorbed, especially if auxiliary agents enhance the transfer from food and non-food particles to biotic ligands as in the gut of organisms (Figure 2.3). Even if the amount of metal accumulated from the aqueous phase is known, one might additionally need to know about the interactions of an organism with metal-contaminated particles in its environment. In principle, the focus on the free ion concentration is risky due to the fact that small lipophilic metal complexes can cross a biological membrane by diffusion. Theoretically, this uptake is under the control of diffusion as mechanistic process, and thus makes the uptake rate very slow. However, in nature this procedure could be the main uptake route if a long term exposure is the case.

2.4 Conclusions

There is general agreement, that in environmental risk assessments the uncertainty will be reduced and the ecological relevance will be increased if both effect and/or exposure data are normalized to the bioavailable fraction by incorporation of bioavailability concepts. However, bioavailability is in nature a complex process, because site-specific geochemical conditions control metal speciation. In addition, physical and biological interactions determine the exposure of organisms to metals associated with soils and sediments, because environments without solids to those metals preferentially are binding is not the general situation. This means, we have to consider the possibility that particle-bound metals are partly bioavailable and contribute to toxic effects, because environmental availability refers to the ability of a metal to interact with other environmental matrices and undergo various fate and transport processes. Therefore the main question as formulated by Fairbrother et al., (2007) is: What are the relevant exposure pathways for inorganic metals to humans and ecological endpoints?

The above-mentioned question is somewhat in contrast to the assumption that barriers must be overcome for a metal in soil or sediment to become bioavailable (Ehlers and Luthy, 2003). If exposure data are normalized to the bioavailable metal fraction by incorporation of bioavailability concepts then approaches were used where restrictive processes are expected to reduce the concentration of metal ions in solution. Examples are the Biotic Ligand Model (BLM) and the SEM-AVS concept (SEM= Simultaneously Extracted Metals; AVS = Acid Volatile Sulfides). Especially the latter concept is under discussion because it is often applied to sediments with a very low acid volatile sulfide fraction where most of the metals interact with the natural organic matter present in the particles. In these cases, it is still difficult to predict the proportion that is bioavailable (Di Toro, 2005). Therefore we think it would be appropriate to extend the scope of bioavailability concepts focused on free ion concentrations towards particle associated metals. Although not often connected with enhancing bioavailability, solid-associated metals are part of integral fate and- transport processes that can control an organism's overall exposure. Then, if metal release from the solid phase occurs internally (as in the digestive system), particles could act as carriers for metals contributing to uptake processes. Even more, this exposure pathway could be the most important, if biological surfaces (e.g. microorganisms) have direct contact with contaminated solids (Liss and Ahlf, 1997).

In general, exposure assessment issues are considered separately for human health, aquatic, and terrestrial receptors, and summarized in a generic conceptual model for metals risk assessment (Fairbrother et al., 2007). After organisms have taken up metals into the cell interior a second distribution process within the organisms influence the internal exposure. Thus means, redistribution of metals within tissues, blood or plasma, or other extracellular space occurs after the initial absorption phase in the body. Organisms are able to control metal concentrations in certain tissues of their body to minimize damage of reactive forms of essential and nonessential metals (Vivjer, 2004). Of particular importance is the bioaccumulation of contaminants within tissues that are often inaccessible to normal elimination mechanisms, such as metabolism and excretion. Slow release of the chemicals from these storage sites can cause protracted "exposure" within the body even when the external exposure has been reduced. In addition, metals may become available at some point to higher-trophic organisms that eat the plant or animal in which the contaminants are stored. Nevertheless for decades the exposure of dietborne metals has been considered to be low for simple organized organisms such as invertebrates (Borgmann et al., 2005). In the last years it has become increasingly evident that uptake of trace metals from the diet may be the major source of metals for many aquatic invertebrates (Rainbow, 2007). Consequently, current regulations should be modified to account for the bioavailability and toxicity of dietborne metals. In our opinion it is time to consider the importance of this exposure route in a reasonable manner.

3 Different Exposure Routes and Regulatory Concern

Environmental quality criteria are the primary benchmarks against which environmental pollution is measured. Although in nature dynamic systems are dominant like in a river basin, where interactions between different compartments (river-sediment-soil-groundwater) greatly influence the ecological quality, criteria were developed for those compartments separately. For example, adjustment of aqueous metal concentrations for differences in water hardness was among the first computational methods to account for bioavailability differences between the laboratory and the field when applying EPA water quality criteria. Currently the relationship among exposure, bioaccumulation, and toxic effects (i.e., critical body burden or organ concentrations) need to be clearly established if these models are to be used for regulatory purposes. Therefore it is useful to discuss the most important uncertainties for water, sediment, and soil in particular.

3.1 Water

The application of the bioavailability concepts to the water compartment consists of the translation of the conventional estimated effect thresholds (i.e. PNEC value, water quality criteria) towards the free metal ion activity in certain surface waters. The most common approach to calculate the bioavailable concentration of a metal is to use a speciation model (e.g. SCAMP/WHAM), whereas the physicochemistry of the surface water has to be known. The development of the biotic ligand model was an improvement, because it considers geochemical and the biological complexities that affect metal exposure. The model combines the influences of speciation (e.g., free metal ion, DOC complexation) and cationic competition (e.g., K+, Na+, Ca2+, Mg2+) on metal toxicity in fish (De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2004; Di Toro et al., 2001; Santore et al., 2001; Deleebeeck et al., 2007). The biological surface (e.g. gill) is seen as a competing ligand in the water phase from a chemical point of view. Recently, this kind of equilibrium partitioning method has become a tool for expanding regulatory use (US EPA, 2003; Reiley, 2007; MERAG 2007). However, significant uncertainties, exceptions, and inherent limitations have been documented numerous to date.

Hassler et al. (2004) have summarized limitations of the models in well-controlled laboratory systems with the goal of extrapolating the results to complex environmental systems. For example, BLM assumes stable systems at equilibrium and consequently regulated metal uptake (e.g. Zn) is not under the scope of this model. In contrast to the limitations described the model was validated in a couple of studies. The situation is less confusing, if we consider those cases, where indeed the free ionic form is most responsible for toxicity.

For regulatory purposes a generic model will be required and there are doubts that the BLM could fulfill such needs. Previously studies demonstrated that metal ions have been directly linked to toxicity in fish and invertebrates while a metal complexed by dissolved organic matter does not induce toxicity to the same degree due to the reduced availability (Ma et al., 1999; Erickson et al., 1996). These forms, or species, were of key importance in understanding bioavailability, and the model modifications were driven by the intention to fit these metal species with their toxic effects. De Schamphelaere and Janssen (2004) reported that acute Cu toxicity to Daphnia magna in Swedish lake water was much higher than predicted from BLM. This discrepancy was ascribed to unusually high levels of Fe and Al, in combination with low pH of 5.5. In general, those site-specific conditions are not within the range of the model and it has to be adjusted for temporal or spatial variability. In most cases uptake of metals bound to DOC is reduced, it is not reversed entirely and can contribute to the total metal burden of an organism and subsequent toxic effects (McGeer et al., 2002). In addition, the main uptake route of metals could be the complexed form from the water phase (Martin et al, 2007). The conceptual model (Figure 2.3) includes this exposure route, considering a slow uptake rate and consistently the exposure time has probably an important influence.

The BLM model assumes the free ion concentration as the most relevant exposure pathway for metals, but there is an increasing perception of the importance of metal complexes for additional uptake. Accordingly we need better insight into the relative bioavailability of the different forms of metal as well as their specific behaviour in nature. Improvements in the detection techniques for determining low free metal ion and labile bound metal concentrations provide tools for a validation of the concept. One study has shown that the labile metal fraction correlated better than the free ion concentrations with toxicity in organisms (Martin, 2007). One explanation could be deduced from our conceptual model taking into account particle-associated metals. This means that in the undisturbed water layer near the membrane the uptake of metals is limited by diffusion processes. After elimination of metal ions in the undisturbed water layer the diffusion gradient is steepened and desorption of labile bound metals from particles could contribute to the bioaccumulation. The proposal is in agreement with the observation, that metals can be transferred between sedimentary phases under unstable situations, which are common in the environment, e.g. in mixing zones (Calmano et al., 1988). The steady-state biouptake of metals from complex media was outlined for the case of two different uptake routes already by Van Leeuwen and Pinheiro (2001). The study comprises the limiting situations of inert and labile complexes, and distinguishes between bioinactive and bioactive (lipophilic) complexes. A conceptual model of some of the important physicochemical processes leading to and following the uptake of a trace metal by an aquatic microorganism is given by Worms et al. (2006).

Again focussing primarily on the main exposure routes of metals in aquatic systems, any Free Ion model alone is not adequate to describe metal uptake and toxicity. Deviations from such models already have been documented e.g. in adult bivalves, accumulating metals not only from water but also from algae, bacteria, colloidal matter, and dissolved organic matter (Roditi et al. 2000, Guo et al. 2001, Lorenzo et al. 2005, Sanchez-Marin et al., 2007). But it remains to be further elucidated whether exposure from additional up-take is more important than from the water phase alone.

3.1.1 Dietborne metals uptake

The underestimation of the dietary exposure is attributed to an inherent failure of studies with spiked sediments, because the pore water concentration in such experiments are unrealistic high (Schlekat and Luoma, 2000). Current studies demonstrate that dietborne metal uptake can significantly influence total uptake, depending on the food source, animal, metal, and exposure conditions (Griscom et al., 2002; Rainbow and Wang, 2001). Wang and Ke (2002) demonstrated that dietary exposure is a dominant source for Cd and Zn accumulation in the predatory gastropods. Consequently, measurements of metal concentrations in the gastropods may only indicate the bioavailable metal level in the prey organisms, instead of the bioavailable metal concentration in ambient seawater. The relative importance of different exposure pathways depends on the BCF in the prey organism, the type of prey organism and the ingestion rate of the predator. Using a medium ingestion rate and a medium BCF in the prey organism, a model predicts that >98% of Cd and Zn in the fish studied are derived from the dietary source when copepods are the prey organisms (Xu and Wang, 2002).

Radiotracer techniques were used to quantify the assimilation and subsequent efflux of silver, cadmium, iron, mercury, thallium, and zinc by mesozooplankton fed ciliates, heterotrophic dinoflagellates, or heterotrophic flagellates, and the results were compared with published values measured for phytoplankton prey. The subcellular distribution of the metals within the prey cells was also determined and related to their bioavailability. The results suggest that metals contained within some protozoa are more efficiently assimilated by copepods than metals in phytoplankton. The higher bioavailability of some metals was correlated with a higher fraction of metals in the cytoplasmic, or liquid fraction of the protozoa (Twinning and Fisher, 2004). Metal compartmentalization in organisms may depend on the primary route of uptake, through the diet or across the epidermal surface, because uptake routes influence not only the total uptake but also the sequestration of metals. When assessing effects, it is insufficient to consider whole body metal concentrations without knowledge of tissue concentrations within the organism. After uptake of the metal (transport across the plasma membrane), the free reactive metal will circulate through the body fluid. Transport proteins will bind the metals reversibly and transport them into different compartments, where they are detoxified or not (Vivjer, 2004). Finding a link between external and internal exposure is one of the challenges in environmental toxicology and offers a great potential for risk assessment (Escher and Hermens, 2004).

Trophic transfer of metals from primary producers to primary consumers is presented in a study, in which a minimal biomagnification of cadmium and copper from algae to *C. dubia* was observed (Sofyan et al, 2006). However, minimal or no apparent biomagnification does not necessarily mean lack of trophic transfer, because metal transfer between trophic levels can still be observed without increasing metal concentrations in the higher trophic levels. It was shown, that the trophic transfer of cadmium and copper was significantly different to the control. This transfer may be sufficient to produce toxicity to higher trophic levels. Because the current water-quality criteria assumes that metal toxicity primarily occurs via the dissolved phase, the finding that dietary cadmium and copper were toxic may have consequences for determining the water-quality criteria of cadmium and copper. Whereas reproduction was observed to be the most sensitive endpoint, survival was observed to be the least sensitive endpoint. These results demonstrate the potential importance of dietary exposure for consideration in metal regulations or risk assessment.

The first study which explicitly demonstrated that Cd can be magnified along certain food chains in nature was presented by Croteau et al. (2005). Trophic enrichment of Cd increases the vulnerability of consumers at the highest trophic levels. However, the Cd source for organisms from higher trophic levels is not easy to find within complex food webs. For some metals analysing ecosystem complexity will be necessary before the species most exposed and at risk can be identified. In the Cd-contaminated environment described it was found that the biomagnification took place among species ingesting Cd-rich epiphytes. The results suggest that to accurately predict Cd biomagnification in nature, physiological biodynamics, habitat, food web structure, and trophic position have to be considered. These factors seem to provide an initial ‘‘set point’’ at lower trophic levels that determines the concentration from which Cd transfers up the food web.

Understanding those conditions will allow a greater appreciation of whether ingested metals need to be considered when developing regulatory guidelines designed to protect aquatic animals. It is about time that dietborne metal exposure will be explicitly incorporated into regulatory frameworks even though the links between exposure and effects are not fully understood. However, the bulk metal concentration of a compartment leads to an overestimation of risk not considering bioavaiability of metals, but if accumulation of metals from water and food is additive, assessments of exposure from waterborne metals could underestimate the metal dose to which aquatic animals are exposed. In particular benthic animals usually accumulated metals mainly through direct ingestion of sediment, regardless of organic or inorganic content. In one study bivalves assimilated up to 20% body burden of Cd from metal sulfide or oxide particles (Lee et al., 2000). The latter may constitute an important particle type eaten by benthic animals.

3.2 Sediment

There is an overall agreement that the bioavailability of metals in sediments is directly influenced by the environmental geochemistry (Lu et al., 2005). The equilibrium partitioning approach (EqP) assumes that chemical activity in the sediment, as indexed by chemical concentration in the interstitial water, is proportional to the chemical’s bioavailability to sediment-dwelling organisms. In anoxic sediments, a key partitioning phase controlling cationic metal activity and toxicity in the sediment-interstitial water system is sulfide (Ankley et al. 1996). The magnitude of reactive sulfide is quantified by measuring the amount of sulfide released into the gas phase when sediment is extracted with 1 N HCl and is known as acid-volatile sulfide (AVS). The determination of metal concentrations from the same extraction procedure referred to as simultaneously extracted metal (SEM). The underlying assumption is that the potential bioavailability of a metal could be predicted by comparing the relative molar concentrations of the SEM and AVS. If sufficient sulfide exists to bind all SEM, then metal toxicity is not expected. Use of this SEM-AVS ratio as exposure estimates that are correlated with toxicity of metals in sediment has been explored closely for many metals (Berry et al., 1999; Burton et al., 2005). In contrast to these studies uncertainty is increasing whether the AVS approach is universally applicable. Lee et al. (2000) pronounce, that the AVS-based approach may be appropriate for protecting some benthic organisms from acute toxicity associated with exposure to very high porewater metal concentrations in extremely contaminated sediments.

We think, that at least in a regulatory framework this sediment approach is overvalued. At first a practical problem has to be solved because AVS concentrations are generally lower in the surface sediments, while SEM concentrations slightly decreased with the depth (Liu et al. 2007). When monitoring environmental impacts by using AVS, both AVS and SEM concentrations in different sediment layers should be taken into account as well as a strong spatial dependence due to differences in sediment composition, total sulfur pools, and redox potential (Eh), which resulted in crucial differences in trace-metal availability within the area studied. The spatial pattern of SEM-AVS could deviate from total or normalized trace-metal patterns, and it is complicated to collect representative samples. The samples should be representative for where the benthic fauna live at ease.

Even if the measurement would be performed correctly AVS is not the sole partitioning phase for predicting sediment toxicity. Results were recorded in contaminated sediments differing in redox potential, AVS, dissolved sulfides, and organic contents, suggesting that other metal ligands are important for metal bioavailability and toxicity in anoxic and suboxic environments besides AVS (Sundelin and Eriksson, 2001). In addition, if we focus the analysis on the biological active layer of the sediment the uncertainty of the AVS approach is growing, regarding the relevance of the method for benthic species with different burrowing behaviour. More concentration on the significance of other sediment characteristics like organic carbon in sediment and pore water, oxihydroxides, salinity, and sediment processing such as bioturbation is needed.

The major problem is that anoxic sediments are toxic for benthic organisms, because nearly all animals require oxygen, with the exception of specific animals which use strategies to obtain their essential oxygen from other compartments. Burrowing into sediment provides a means of avoiding predators; pumping oxygen-rich water through a burrow allows an animal to live at the interface of oxic and anoxic compartments. The relative importance of the water column and sediment compartments as metal sources for a given species is likely to be influenced by the form of its burrow and the composition of the burrow wall as well as by the rate at which the animal irrigates its burrow. Results presented by Hare et al. (2001) strengthen the argument that the protection of benthic communities from metal pollution should consider metals in both the water column and sediment compartments. In this regard, the AVS model, which considers only sedimentary metals, was more effective in predicting metal concentrations in pore waters than those in most animal taxa. Metal concentrations in pore waters were lowered in oxic sediments possibly because of their association with Fe-oxy-hydroxides or sediment organic matter (Lu et al, 2005).

It should be noted that there is no answer to the introducing question for the main uptake routes of metals to biota in sediments from the SEM-AVS concept. Furthermore the use of organic carbon and other ligands to normalize the total metal concentrations in pore water is a physico-chemical correction and do no not represent the true bioavailable fraction. As for the compartment water the effect of competition with biotic ligands could be taken into account. In this regard an extension of the SEM/AVS procedure was presented that predicts the acute and chronic sediment metals effects concentrations (Di Toro et al., 2005). A biotic ligand model (BLM) and a pore water–sediment partitioning model are used to predict the sediment concentration that is in equilibrium with the biotic ligand effects concentration. The initial application simplifies the complex situation and considers only partitioning to sediment particulate organic carbon in competition with organic biotic ligands.

However, as well as sediment properties influencing metal partitioning (*K*d), the speciation of the particulate phase (e.g., sulfides, organic matter, iron hydroxides) will also influence the assimilation of ingested particles. With respect to sediment exposure pathways, the degree of assimilation of each metal from each sediment phase will depend on the organism’s physiology (e.g., gut passage time, gut chemistry) as well as the properties of the sediment phase. The metal assimilation efficiency (AE) of the organism affects the exposure an organism receives from particulate contaminants that are accumulated through the digestive system (Simpson and King, 2005). The use of AVS assumes that the sulfides limit bioavailability universally, which may not be true, if animals oxidize sediments in their gut. The same processes occur in nature at the interface of sediment and water column, where seasonal dynamics generate a rapid exchange of toxic compounds between the top layer of the sediment and the overlaying water (Hsu et al., 2007).

The equilibrium approach has increased our understanding of some geochemical processes in sediments, but pore water should not be presented as the only available source for organisms living in or on sediments. Alternative methods might be to relate exposure to the metal content in overlaying water, or to make bioavailability corrections, similar to what is proposed for water. The most common regulatory and scientific challenge is the need to determine the ecological implications of sediments contaminated at less than extreme levels. Thus means, pore water, overlaying water, particles in general, and dietborne metals have to be considered as potential uptake routes of metals for benthic organisms. Important uncertainties in the application of the AVS-equilibrium concept exclude this approach for the prediction of bioavailable metal exposure concentrations as either a regulatory tool or a scientific generalization.

3.3 Soil

For implementing bioavailability into the exposure assessment of metals in soil, two main issues are used. First, a translation of exposure from total to pore-water or free-ion concentrations, and second a soil type-correction of toxicity data. For converting total concentrations to pore-water or free-ion concentrations, different speciation models are proposed. This approach is based on the assumption that impacts on test organisms (plants, micro-organisms and soil invertebrates) are mainly related to the soil solution concentration and not to the soil solid phase content. Critical Cd, Pb, Cu, Zn and Hg concentrations in soil solution vary with pH and DOC level. The results obtained are generally comparable to those derived for surface waters based on impacts to aquatic organisms. Critical soil metal concentrations, related to the derived soil solution limits, can be described as a function of pH and organic matter and clay content, and can vary about one order of magnitude between different soil types (de Vries et al., 2007). The supposition is that the pore-water is the main exposure route for organisms and that a point estimate of exposure reflects long-term toxicity. This could be true for plants, root uptake is the most important with leaf exposures secondary, with the exception of Hg where the majority is accumulated via foliar uptake. Consequently a Terrestrial Biotic Ligand model (TBLM) was developed, in which soil organic matter is the partitioning phase for metal in soil. Free metal ions react with the biotic ligand, the receptor site, and inhibit root elongation (Thakali et al., 2006a). An autovalidation of the BLM indicated that predicted EC50s differed from the observed EC50s by a factor of less than 2, indicating that the BLM concept may be used to predict metal toxicity to terrestrial plants (Lock et al., 2007). Moreover zinc toxicity to nitrification in soil and soilless culture were compared with the same Biotic Ligand Model and the Zn2+ activities resulting in 20% reduction of the nitrification. These inhibitions were well predicted using the same parameters for both (soil and soilless) systems, indicating that microorganisms in soil are exposed to zinc through the free zinc ion in soil pore water (Mertens et al., 2007).

There are a few fundamental objections against an equilibrium approach, because most plants and biota will accumulate many times the amount of metal available in solution at any given moment. In effect the soil or sediment solution is emptied and replenished. Metal uptake is, thus dependent not only on the availability of the metal in solution (intensity factor) and the uptake mechanisms, but also on the capacity of the soil or the sediment solid phases to supply the fluxes of that particular element (capacity factor). The chemical processes of ion exchange, adsorption, precipitation, diffusion, volatilization and complexation determine the physicochemical form or chemical speciation, of metals in solution. The impact of these processes on the activities of the free metal ion in solution further depends on the composition of the aquatic matrix, and jointly they determine the concentration of the metal ion in solution. The master variable affecting metal speciation is usually pH. For instance, metals tend to be more soluble as pH decreases. Elemental uptake is controlled by chemical availability in solution as well as the capacity of the soil, sediment, or labile fractions present in solution to supply that element. Understanding bioavailability for species exposed through the pore water requires a consideration of both aspects: the intensity of exposure through the FIAM and the capacity of the soil or the sediment to maintain this level of free-metal activity in solution (Peijnenburg and Jager, 2003). A simple extraction of metals from the soil solids does not reflect this dynamic process. Shown in a study concentrations of Pb, Ni, Cu, and Cd in roots and leaves of *Dactylis glomerata* were unrelated to the total or DTPA-extractable concentrations in a sludge amended soil (Ortiz and Alcaniz, 2006). Authors of a literature survey concluded that according to current knowledge, bioavailability cannot be included in risk assessment schemes (Frische et al., 2003). The biotic parameters are related to contaminant concentrations after non-exhaustive chemical extractions, alternatively to values calculated by theoretical models such as the equilibrium partitioning theory. A general problem of all methods is that the spatial and temporal components of the bioavailability concept are hardly taken into account (Frische et al., 2003). However, recent studies indicate that a terrestrial BLM may work for Cu in one earthworm species (Steenberger et al., 2005). In laboratory studies with bioassays Cu and Ni toxicities were incorporated to multiple endpoints associated with higher plants, invertebrates, and microbes for up to eleven noncalcareous soils of disparate properties, into a single theoretical framework. The results of this study clearly demonstrate that the TBLM can provide a general framework for modeling metals ecotoxicity in soils (Thakali et al., 2006b).

The EqP concept is, however, less suited for predicting possible adverse effects for organisms whose predominant exposure route is not the pore water, or for (an exposure route) in which the bioavailable fraction is not in equilibrium with the metal fraction in the pore water. For earthworms the exposure route is not clear, and may be compound dependent (Scott-Fordsmand et al., 2004). In addition to pore water, the major exposure pathways of metals to soil-dwelling organisms are ingestion, with a minor and often unknown dermal uptake component. Nevertheless soil invertebrates are assumed to be exposed through direct contact to contaminated particles. Much work has been carried out on accumulation of metals by earthworms in soils and how it is affected by metal concentrations, soil properties and time. Yet it is still not possible to predict with a high degree of confidence the body burden of an earthworm at a contaminated site (Nahami et al., 2007).

A couple of principles of metal transport and fate in soils are applicable to all terrestrial organisms and should be mentioned briefly. The amount of positively charged cations a soil can hold is described as the cation exchange capacity (CEC). CEC is strongly dependent on the type and amount of organic material (OM) and oxyhydroxides present in the soil, and is strongly pH dependent. Surface charge on OM and oxyhydroxides increases with pH, thereby increasing their sorptive capacity for metals In general cationic metals are more bioavailable at lower soil pH and less bioavailable at higher soil pH, if pore water is the main pathway of uptake. The key soil parameters pH, CEC, and organic carbon tend to be less important for soil if exposure based primarily on diet and incidental ingestion of soils. Food and the incidental ingestion of soil are the two most important exposure pathways for terrestrial wildlife. The relative importance of exposure pathways (soil vs. diet) is determined by the fraction of metal-contaminated soil in the diet and the concentration of metal in food items, whereas uptake from soil into soil invertebrates is a relatively more important pathway than for animals on a higher trophic level (Fairbrother et al., 2007). The importance of the gut and its contents contribute significantly to the body burden and it was recommended to measure the metal load in the gut when modeling food-chain transfer from small mammals to their predators (Walker et al., 2002). However, there are other factors complicating the laboratory-to-field extrapolation.

Under field conditions, the toxicity of a substance may clearly differ from effects found in laboratory experiments (e.g., Smolders et al., 2003). In studies soil from a contaminated field site was brought into the laboratory and tests were preformed with both newly spiked soil (soil from the site) and with field contaminated soil. Tests were performed with collembolans and earthworms. Exposing these organisms to Cu in the field contaminated soil indeed resulted in lower toxicity values than see from exposure in newly spiked soil. The discrepancy between spiked soil and soil from the Cu polluted site reflected that copper was less available to the plants in the field also (Scott-Fordsmand et al., 2000 a,b, Strandberg et al., 2006). This may be due to “ageing”, a bonding process of a contaminant to soil particles resulting in a lower bioavailability of the chemical to a terrestrial organism. In nature, effects of diverse 'aging' or 'diagenetic' processes occur after deposition of the solid matter. For inorganic pollutants, mainly heavy metals and arsenic, the process of aging predominantly consists of an enhanced retention. The involved mechanisms are sorption, precipitation, coprecipitation, occlusion, and incorporation in reservoir minerals, governing the rate and extent of decline in bioavailability (Ahlf and Förstner, 2001).

Bioavailability considerations are one of the tools for a proper assignment of sites potentially and actually at risk as it allows assessing in a more reliable way the extent of exposure. In a paper, bioavailability considerations are linked to physicochemical methods available for assessing metal fractions in soils (Peijnenburg and Jager, 2003). Actually or potentially occurring adverse effects of either excess or shortage of (essential) metals are species dependent, metal dependent, and soil specific. As most assessment methodologies are operationally defined instead of being functionally defined, examples of mechanistically based monitoring approaches of bioavailability are still scarce. The value of the methods for measuring bioavailability can be significantly improved when the species-, metal-, and soil-specific aspects of bioavailability are more accurately taken into account in the design of chemical simulation methodologies (Peijnenburg et al., 2007). In contrast, direct measurements of bioavailability and bioaccumulation of metals in soil living organisms in test arrangements could summarize all influencing factors. The coherence of a complex environmental system is already considered in monitoring studies. A contaminated area was assessed for Zn, Cr, Cu, Ni, Co and Pb in soils, forage grass, milk from cattle, leafy and non-leafy vegetables. Partitioning pattern of soil revealed high levels of Zn, Cr, and Cu associated with labile fractions, making them more mobile and plant available. The associated risk was assessed using hazard quotient. Human risk was assessed in people known to consume these contaminated foods by analyzing metals concentrations in venous blood and urine (Sridhara Chary et al., 2007).

4 How Should Regulations Consider Metal Bioavailability?

An important step forward would be to determine the plausibility of the methods applied in existing regulatory guidelines in order to delineate the main uptake routes for metals in different compartments. The goal should be that bulk metal concentrations are adjusted by appropriate bioavailability factors to achieve comparable, actual uptake of metals by organisms. This will standardize exposure values across different types of environments and allow for more accurate comparisons with laboratory toxicity data. The BLM and TBLM approach provide a means to predict ecotoxicological effect of metals on the environment. Their data requirements are small and only routine measurement are needed as model inputs (Allen and Janssen, 2006). However, risks posed by metals in nature depend on exposure, but not from the water phase solely. As shown in Fig. 1 the influence of labile bound metals and metal complexes should be implemented in refined models. Needless to say, predicting the effects of metal contamination is difficult because biological responses differ among exposure routes. For example, the variations in metal accumulation in earthworms could not be explained by any individual field parameter. Risk assessments should therefore be apprehensive to a single stress approach (Vijer et al., 2007).

It is now increasingly accepted in the scientific community that dietary accumulation of metals is at least as important as metal uptake from the aqueous phase and in many cases dominates metal accumulation e.g. in bivalves from marine environments. Models could be improved or even replaced by incorporating dietborne metals. There is increasing perception of biodynamics, because understanding the biological processes that modulate dietborne metal uptake is crucial to assess the toxicity of dietborne metals (Croteau et al., 2007). These models can improve predictions of metal bioaccumulation in aquatic organisms because they incorporate different exposure routes (e.g., water vs. diet) and the dynamic nature of metal bioaccumulation processes. For example, Luoma and Rainbow (2005) reviewed the DYNBAM model (a single-compartment, kinetic-based bioaccumulation model) and found it to accurately predict metal bioaccumulation for a wide range of metals, organisms, and habitats based on data derived from 15 separate studies. Importantly, DYNBAM and similar such models require experimental data measured under environmentally realistic conditions in order to derive model parameters for each metal–species combination (e.g., uptake and elimination rates, assimilation efficiency, food ingestion rates).

A move towards a unifying model was presented with the underlying idea to link the BLM approach with biodynamics. Cadmium influx rates correlate with changes in the affinity of the biotic ligand, whereas those of Cu correlate with changes in both site affinity and capacity. A relationship between metal influx rate and ligand character asks whether toxicity is the result of accumulation at the biotic ligand or the rate at which metal is transported by that ligand (Croteau and Luoma, 2007). Merging knowledge of transport physiology and biodynamics may provide key insights to understand metal bioaccumulation and likely, help to better predict metal toxicity.

Patterns of metal distribution among organs vary depending on whether the metal is taken up from food or water. Cu concentrations in rainbow trout were higher in the gut compared to the gill when metal was taken up from food, whereas concentrations of those metals in respiratory organs were higher when water was the major metals source (Kamunde and Clayton, 2002). For these reasons, it can be useful to separate animals into compartments for modelling purposes. – To understand and predict toxic effects on animals, it might be necessary to consider the bioavailability and effects of intracellular metals on cellular processes.

5 Environmental Risk Assessment of Metals

Following the previous chapter's evaluation of those processes that cause risks for organisms, this chapter will deal with the question of how much of this information is really used in risk assessment that is carried out not by scientists but by stakeholders like environmental authorities. For the quantification of the uncertainty in the extrapolation of the relative toxicity of metals from test organisms to wild life e.g. species sensitivity distributions (SSDs) were applied. SSDs are suitable to visualize a well populated distribution, but the disadvantages of SSDs are that robust results can only be obtained from well balanced (parity), complete data-sets with representative taxa. These problems of toxicity extrapolation and of statistical robustness are not under the scope of the report.

As one of the most ambitious legislations using risk assessment we chose the Water Framework Directive as a case by which we will compare how much of the available knowledge and recommendations on risk assessment is taken up into regulation.

5.1 Basic statements in metals risk assessment

Risk is scientifically defined as the product of the magnitude of possible adverse effects (hazard) and the probability of exposure. The foundation of **risk assessment** is the scientific evaluation of information. The purpose of risk assessment is to summarize and present scientific knowledge so that it can be used to make sound management decisions.

Two extensive reports have summarized the scientific foundations and current knowledge of metal risk assessment in 2007: The *Metals Environmental Risk Assessment Guidance* (MERAG) (ICMM, 2007) which was produced by an international team with a strong European core, and the *Framework for Metals Risk Assessment* of the US EPA (EPA, 2007; Fairbrother *et al.*, 2007). Even though both recently published reports cover almost the same scientific topics, the EPA-Framework is more directed towards practical help in decision making and puts a stronger emphasis on different models, while MERAG is a scientific report with less concrete guidance for practitioners. A technical guidance document gives guidance on the chemical safety assessment of metals under REACH.

Both reports however aim at informing risk assessors and, via these, decision makers. They concentrate on inorganic metallic compounds and stress the following information as the basis for metals risk assessment with the listed consequences:

a) Metals occur naturally in the environment and their concentrations vary between different geographic regions

→ due to different background concentrations, effective concentrations differ between regions due to acclimation or adaptation

→ the risk from metal concentration may be different depending on the region, temporary, geographic and cultural (e.g. nutrition habits) differences (use of metalloregion concept as one possibility)

b) Some metals are essential for humans, plants, animals, or microorganisms and beneficial within an optimal concentration range for essential elements.

→ metal deficiency would act as an additional stressor

→ surplus of essential metals can show inhibitory effects

c) Geochemical characteristics of metals determine their environmental properties and their effects on human and ecological receptors

→ the chemical-species depending effects on bioaccessibility, bioavailability, effects and fate need to be considered

→ site-specific characteristics like pH, particle size, humidity, redox, organic substance, cation exchange capacity may affect bioavailability of metals differently.

d) Toxicokinetic and toxicodynamic properties of metals depend on the respective environmental species and the capability of the organisms to regulate or store the metal

→ bioaccumulative properties that (may) lead to toxic effects need to be considered

→ BCF and BAF-values have to be considered with care.

The EPA report makes a stronger statement than MERAG with regard to BCF and BAF-values. It summarizes that they should not be used as criteria for effect thresholds, but are considered valuable on a site-specific basis under consideration of local ambient chemistry. It is specifically mentioned that bioaccumulation models like DYNBAM which consider kinetic processes have been shown to work well and may have the potential to replace the BCF/BAF approach.

While MERAG does not address the problem of metal mixtures, the EPA-report stresses that additive effects need to be taken into account when assessing bioavailability, that interactions between metals may occur, e.g. when competing for the same ligands, and that the presence and concentration of other metals should be taken into account when interpreting results of toxicity tests.

Furthermore the report emphasizes that internal regulation, the kind of tissue that is affected as well as the exposure route and the physiological state of the organism can all influence bioavailability, bioaccumulation and toxic effects.

While these reports cover the existing scientific basis, the integration of this information in regulatory frameworks remains to be challenging. As an example, the evaluation of metals risks in one of the most important pieces of European environmental legislation in recent years, the Water Framework Directive, will be examined in more detail.

6 The integration of scientific knowledge into regulatory risk assessment – The case of the Water Framework Directive (WFD)

6.1 Introduction to the WFD

The European Water Framework Directive (WFD) (EC, 2000) establishes a framework for the protection of groundwater, inland surface waters, estuarine waters, and coastal waters. It constitutes a new view of water resources management in Europe, based mainly upon ecological elements.

With regard to surface waters, it aims to

a) prevent deterioration

b) achieve a good status of by 2015

c) achieve a good ecological potential and a good chemical status for all artificial and heavily modified water bodies by 2015

d) control pollution at source through the setting of emission limit values and of environmental quality standards (EQS).

The chemical status is determined by currently 33 "priority substances" of the WFD and 8 European wide regulated substances of the 76/464 guideline (now codified as 2006/11/EC). The ecological status comprises additional environmental quality values for specific pollutants which are emitted in significant amounts.

The term "pollution" is defined in Article 2, WFD, as "direct or indirect introduction, (…), of substances or heat into the air, water or land which may be harmful to human health or the quality of aquatic ecosystems or terrestrial ecosystems directly depending on aquatic ecosystems, which result in damage to material property, or which impair or interfere with amenities and other legitimate uses of the environment." Therewith the WFD provides a legal framework for risk management objectives in river basins that addresses the health of humans, the quality of the aquatic ecosystem and those areas that are affected by the rivers (e.g. flood plains), economic aspects, and ecosystem functions such as recreation and fishery.

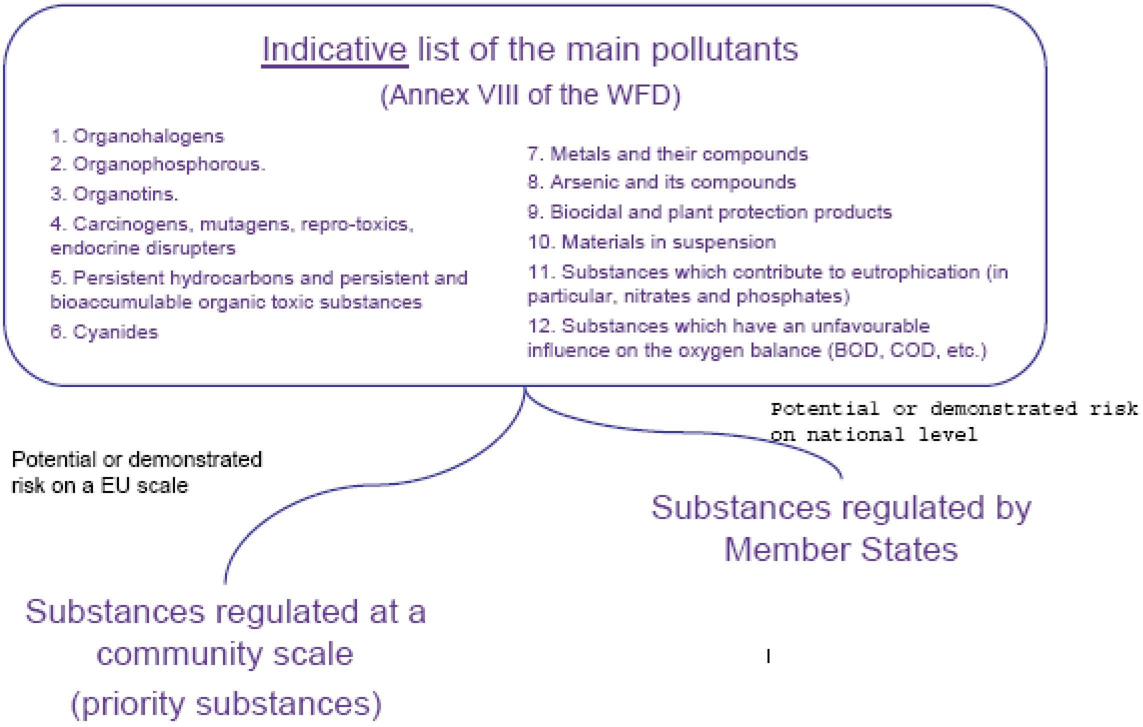
Pollutants of Concern

The main pollutants of concern with regard to risks for aquatic life are in principal listed in Annex VIII, WFD, but their regulation is accounted for differently: Those substances which have been identified on the community level to present a risk to, or via, water and therewith to the aquatic life or human health are classified as **priority substances (PS)**, with the most hazardous of these classed as **priority hazardous substances** (PHS). PS are listed in Annex X of the WFD after adoption by the European Parliament and the Council.

Other substances indicated in Annex VII are going to be regulated on a national scale (Figure 6.1). From thousands of substances that potentially pose an environmental risk, those most relevant need to be selected by each country. The substances that are listed in Directive 76/464/EEC (Council Directive on pollution caused by discharges of certain dangerous substances) (see table 6.1) - now codified as 2006/11/EC - are used here as a starting point.

The Environmental Quality Standards (EQS) are supposed to be based on acute and chronic ecotoxicological data from algae or macrophytes, daphnia or marine representatives and/or fish. Depending on the number and kind of available data, safety factors of between 10 and 1000 are applied to set the EQS unless more precise safety factors can be deduced from field data or model ecosystems, by comparison with field studies or after peer review and consultation.

The WFD in Annex IV 1.2.6 wants available data on persistence and bioaccumulation taken into account, leaving open in which way this can be done. The manual on a methodological framework to derive Environmental Quality Standards for Priority Substances by Lepper 2005 contains more guidance in this issue.



Indicative list of the main pollutants
(Annex VIII of the WFD)

1. Organohalogens
2. Organophosphorous.
3. Organotins.
4. Carcinogens, mutagens, repro-toxics, endocrine disrupters
5. Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances
6. Cyanides
7. Metals and their compounds
8. Arsenic and its compounds
9. Biocidal and plant protection products
10. Materials in suspension
11. Substances which contribute to eutrophication (in particular, nitrates and phosphates)
12. Substances which have an unfavourable influence on the oxygen balance (BOD, COD, etc.)

Potential or demonstrated risk on a EU scale

Substances regulated at a community scale (priority substances)

Potential or demonstrated risk on national level

Substances regulated by Member States

Figure 6.1: Indicative list of main pollutants as listed in Annex VIII of the WFD (modified from V. Bonnomet (INERIS), presentation held at EMEA Conference 27 – 28 October 2005, London)

Priority substances

On 17 July 2006, the European Commission adopted a proposal for a new Directive to protect surface water from pollution (COM(2006)397 final). The proposed Directive, which is required to support the Water Framework Directive, sets limits on concentrations in surface waters of 41 dangerous chemical substances (including 33 priority substances and 8 other pollutants) that pose a particular risk to aquatic life, animals consuming aquatic biota, and to human health. These substances are used to identify the CHEMICAL STATUS of the water body. The proposal also requires progressive reduction of emissions, losses, and discharges of all priority substances, and phase-out or cessation of emissions, losses and discharges of priority hazardous substances within 20 years (http://ec.europa.eu/environment/water/water-dangersub/surface_water.htm). Priority substances are listed in Annex X of the WFD after adoption by the European Parliament and the Council and comprise the following metals: cadmium, mercury, lead, nickel (Organometal compound: tributyltin).

Table 6.1: Frequency of exceedance (2002 – 2004) of a) target values (GLK II); b) nationally determined quality targets according to 76/464 (QK) which up to now are not legally binding; c) Quality targets from 76/464 regulated on community level (EGQZ); d) Quality standards for the ecological status (VO); e) EQS for the chemical status. Draft from July 2005 (EQS = average, MAC-EQS = maximal value) (from Blondzik et al, 2006). Colours reflect exceedance of target values/standards:
🟩 No exceedance; 🟨 exceedance at up to 10 % of locations;
🟧 exceedance at 10 to 25 % of locations; 🟥 exceedance at more than 25 % of locations.

EG-NR	Substance	GKL II (mg/kg)	QK (mg/kg)	EGQZ (µg/l)[1]	VO (mg/kg)	EQS (µg/l)[2]	MAC-EQS (µg/l)[2]
4	Arsenic				🟨 40		
List II	Antimony		🟧 6				
List II	Barium		🟨 1000				
List II	Beryllium		🟩 10				
List II	Chromium	🟧 100			🟩 640		
List II	Cobalt		🟨 80				
List II	Copper	🟥 60			🟧 160		
List II	Molybdenium		🟧 5				
List II	Selen		🟧 4				
List II	Silver		🟧 2				
List II	Tellurium		🟥 1				
List II	Thallium		🟩 4				
List II	Titanium		🟩 10000				
List II	Uranium		🟧 1				
List II	Vanadium		🟩 200				
List II	Zinc	🟥 200			🟥 800		
List II	Tin		🟨 20				
WFD 6	Cadmium	🟥 1,2		🟨 1		🟫 0,08	🟫 0,45
WFD 20	Lead	🟥 100	🟥 100			🟩 2.1	🟨 2,8
WFD 21	Mercury	🟧 0,8		🟩 1		🟩 0.05	🟩 0,07
WFD 23	Nickel	🟥 50	🟨 120			🟥 1,7	🟨 20,4

Substances regulated by Member States

Quality targets ("QK" in table 6.1) of the Directive 76/464/EEC (now codified as 2006/11/EC) serve to determine the ECOLOGICAL STATUS of the water body, and have been transferred into the federal regulations of the LÄNDER in Germany. For substances accumulating in sediments and

---

[1] Whole water sample

[2] Filtered water sample

suspended particulate matter (SPM), quality standards have been determined for the matrix "suspended particulate matter" in Germany (VO, figure 6.2), assuming a better analytical determination due to the higher concentration compared to the water phase. The quality standards for metals in SPM were politically set as a multiple (4 to 8) of the background concentration. This decision was made, because the lowest NOECs of many metals are close to the background concentration, and because background values differ regionally. EQS for the determination of the chemical status have been developed for dissolved concentrations after filtration of the water sample.

Table 6.1 shows the regulations for different metals and the frequency with which the target values/standards of 2006 have been exceeded in rivers in Germany between 2002 and 2004 (Blondzik *et al.*, 2006) whereby the EQS and MAC-EQS standards for cadmium, lead and nickel have been changed since then and only have informative meaning here. The new standards for these substances are described in chapter 6.1.2 and shown in Table 6.2.

When focusing on mercury, for which the EQS are still the same, a comparison of exceedances of the target values/standards measured in suspended matter (GKL II; QK; VO in Table 6.1) with those measured in the filtered water samples (EQS, MAC-EQS) makes apparent that different conclusions on risk would be drawn: the information gained from exceedances of target values would range from "no risk" due to mercury at all sites with filtered samples, to an existing risk at 10 to 25 % at the measured locations when addressing SPM (See chapter 6.3).

6.1.1 Deriving Environmental quality standards

EQS-development was based largely on current European Technical Guidance Documents (TGD) used in chemical risk assessment (EC, 2003) to derive Predicted No Effect Concentrations (PNECs). A special guidance document for the derivation of EQSs was developed on the basis of the TGD by the Fraunhofer Institut (FHI) on request of the European Commission (Lepper, 2005) (see Figure 6.2). The EQSs to protect aquatic life are based on the lowest reliable toxicity test No Observed Effect Concentration, or the lower 5th percentile from a species sensitivity distribution.

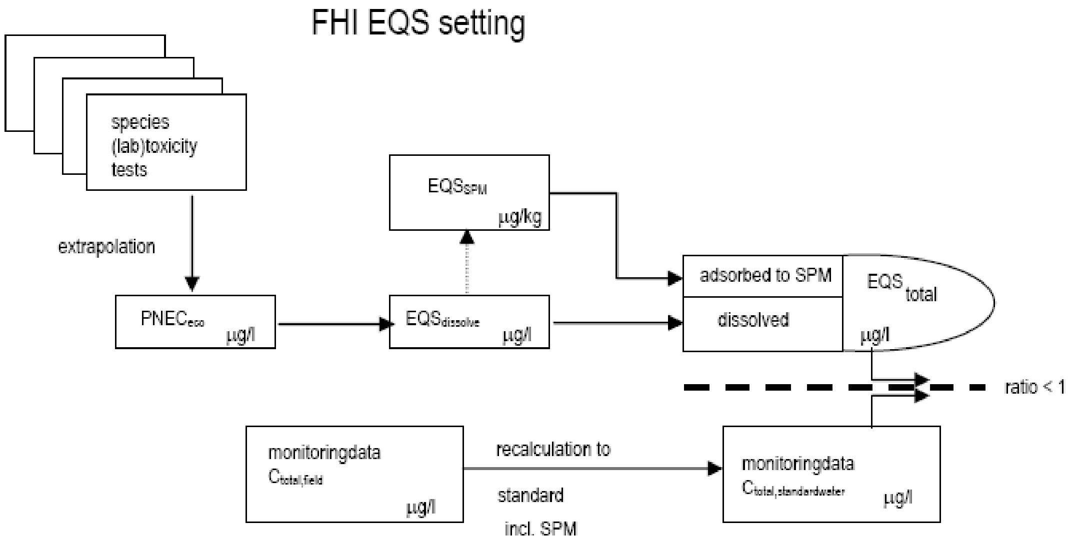


Figure 6.2: Dissolved and total water concentrations in TGD risk assessment and FHI EQS derivation (Anonymous, 2003)

An assessment factor is applied to derive a PNEC which is applied as EQS and assumed to be protective of all potentially exposed species. Two types of water column EQS were proposed for each priority substance: an annual average to protect against relatively low-level, long-term effects and a maximum acceptable concentration (MAC) to protect against relatively high-level, short-term effects, where applicable. For naturally occurring substances, such as metals, FHI recommended use of the “added risk approach”, in order to take background concentrations into account.

6.1.2 The integration of FHI- Guidance into the development of EQS for the WFD under consideration of the scientific basis that has been pointed out by MERAG / EPA-framework

a) Natural and geographically different occurrence of metals in the environment

Lepper (2005) adapted in the FHI-report the Dutch “added risk” approach for derivation of quality objectives of metals and other naturally occurring substances as this facilitates to account for natural background concentrations and therewith to avoid setting regulatory standards below the background concentrations. This concept is based on the assumption, that species in an environment with naturally increased metal concentrations have adapted to this situation, and that any additionally added metal concentration affects these organisms the same way as non-adapted. The concept is simple: a maximum permissible addition (MPA) to the background level of a certain metal is calculated from toxicity studies. The MPA is the amount of metal that may maximally be added to the background concentration of this metal without adversely affecting the assessed ecosystem. The MPA_{water} refers to the "bioavailable" (using dissolved fraction as the best available approximation) metal [µg Metal / l] and is calculated either on the basis of long-term NOEC data (e.g. by the species sensitivity distribution method or by the assessment factor method). The corresponding MPA_{SPM} for suspended particulate matter (SPM) [µg Metal / kg SPM] is calculated based on the MPA_{water} and the locally relevant $Kp_{water-SPM}$ ($MPA_{SPM} = MPA_{water} * Kp_{water-SPM}$)

The background concentration is either added as concentration in water or as concentration in SPM in order to derive an EQS_{water} or a EQS_{SPM}.

$EQS_{water} = C_{background.water} + MPA_{water}$

$EQS_{SPM} = C_{background.SPM} + MPA_{SPM}$

The EQS’ that the Commission finally decided upon, however, are based on the total risk approach, which accounts for the total dissolved amount of a metal in a water body without any distinction being made between the fraction of a metal that is present in a water body for natural reasons and the fraction added due to anthropogenic activities. The assumption behind using the total risk approach is, that also background concentrations interact with the organisms. As a consequence and considering the assessment factors which have been proposed by the TGD (and by which the lowest NOEC is divided to account for uncertainties in the transfer to the ecosystem), this may result in some EQS being below the natural background concentrations.

b) Requirements for essential metals

An “added risk” would prevent that EQS fall below the optimal concentration range for essential elements as these would be covered by the background concentrations.

This, however, could be a problem, if EQS which derive from the total risk approach are used.

c) Characteristics of metals and site-specific parameters influence the effects of metals on human and ecological receptors.

The FHI-Report states that for most metals, not enough scientific knowledge is available to predict metal bioavailability to different aquatic life forms in relation to modifying influences of water quality parameters.

In order to address differences in bioavailability, EQS refer to the “dissolved” rather than to the total concentration of metals in water, under the assumption that especially the metal ions and other dissolved species are available and therefore relevant for toxicity while the total concentration of the metal in question may include a significant non-bioavailable metal fraction (e.g. sequestered or adsorbed to particulate matter) (Lepper, 2005).

The WFD also requires determination of metal concentration in the dissolved fraction (filtrate <0,45 µm) instead of in the total fraction. Influence of physico-chemical parameters on metal bioavailability has found no consideration in setting quality standards up to now. The only exception is cadmium as for this metal an (approximate) assessment of bioavailable concentrations might be possible. A regression function based on increasing chronic toxicity values of several test organisms (*Daphnia magna*, *Pimephales promelas* and *Salmo trutta)* with increasing water hardness has been established recently and is suggested in the ongoing cadmium risk assessment for risk characterization at a local or regional scale (Table 1).

d) Toxicodynamic and Toxicokinetic properties depend on the respective chemical species and on the physiological capability of the organisms to cope with the exposure.

Biota-EQS are only proposed for 3 of the 33 priority substances, despite the fact that 18 of 33 have a K_p of more than 3 and therewith potentially the ability to bioaccumulate (Heise et al, in print). Of these 3 Biota-EQS, Mercury and its compounds is the only metallic compound for which an EQS for concentrations in biota is considered (max. 20 $\mu g\ kg^{-1}$). In line with the recommendation of the CSTEE, the FHI-report suggests to base the assessment for secondary poisoning of top predators on methyl mercury which is much more toxic and abundant in organisms than Hg. Predators such as mammals and birds feed on prey (fish, mussels) that may contain mercury of which 70-99% is assumed to be organic mercury.

The EQS for secondary poisoning then derives from the following quotient: NOECs for effects on birds and mammals (0.22 mg/kg) divided by an assessment factor (AF) of 10 as many NOEC-data are available (otherwise AF would have had to be 30).

Lepper (2005) suggested to derive Biota-EQS for those organic substances and metals with experimental BCF ≥ 100 or BMF >1. He agrees, however, that BCF/BAF data are not reliable as they depend on metal concentration in water. According to the US EPA, BAFs generally range up to two orders of magnitude even within one trophic level due to various site specific biotic and abiotic factors (from Environmental Quality Standards (EQS); Substance Data Sheet; Priority Substance No. 21). Lepper hence proposes not to use BCF values of studies conducted in waters with extremely low (i.e. lower than in the upper range of background levels) or high metal concentrations for the calculation of quality standards.

TABLE 6.2: PROPOSED WATER FRAMEWORK DIRECTIVE ENVIRONMENTAL QUALITY STANDARDS (EQS) FOR THOSE METALS THAT ARE LISTED AS ANNEX X PRIORITY SUBSTANCES (HIGHLIGHTED IN GREY= PRIORITY HAZARDOUS SUBSTANCE). AA = ANNUAL AVERAGE (ARITHMETIC MEAN), MAC = MAXIMUM ALLOWABLE CONCENTRATION. ALL VALUES ARE IN MG L^{-1} DISSOLVED CONCENTRATIONS (EC 2006).

Name of substance	**CAS number**	**AA-EQS Inland surface waters**	**AA-EQS Other surface waters**	**MAC-EQS Inland surface waters**	**MAC-EQS Other surface waters**
Cadmium and its compounds *(depending on water hardness classes)*	7440-43-9	≤ 0.08 (Class 1) 0.08 (Class 2) 0.09 (Class 3) 0.15 (Class 4) 0.25 (Class 5)	0.2	≤ 0.45 (Class 1) 0.45 (Class 2) 0.6 (Class 3) 0.9 (Class 4) 1.5 (Class 5)	
Lead and its compounds	7439-92-1	7.2	7.2	*not applicable*	*not applicable*
Mercury and its compounds	7439-97-6	0.05	0.05	0.07	0.07
Nickel and its compounds	7440-02-0	20	20	*not applicable*	*not applicable*
Tributyltin compounds (Tributhyl-tin-cation)	36643-28-4	0.0002	0.0002	0.0015	0.0015

On 17 June 2008 the European Parliament approved the Council Common Position on the adoption of a directive on Environmental Quality Standards of Priority Substances. In this Daughter Directive, biota-EQS' have only been recommended for hexachlorobenzene, hexachlorobutadiene and mercury. . It is, however, explicitly stated, that Member States may opt to apply EQS for sediment and/or biota instead of those laid down in Annex I, Part A in certain categories of surface water. Member States that apply this option shall apply the EQS for bioaccumulation and establish and apply furthermore EQS for sediment and/or biota for specified substances which shall offer at least the same level of protection as the EQS for water. It has, however, not been stated, how these should be derived.

6.2 Quality targets for other media in legislation – soil and sediments

Sediment

While the WFD stated, that standards can be set for water, sediment or biota, no sediment standards were included in the Daughter Directive. If standards were set, compliance monitoring would have to be carried out by the Member States. As recounted by Crane and Babut (2007), who give an excellent report on the discussion of deriving EQS for sediments, the main criticisms for not applying EQS for sediments were concerns about the uncertainty connected with factors affecting, or at least influencing, sediment toxicity as well as compliance checking. The former group of concerns stems from sediment heterogeneity, confounding factors such as ammonia, uncertainties related to assessment or testing approaches, and ultimately to the lack of unambiguous relationships between toxic effect endpoints and (individual) chemical concentrations in sediment (Crane & Babut, 2007).

Following the advice from the EAF PS[3], the AMPS[4] working group proposed in 2004 not to establish quality standards for biota and sediment at this stage, but to designate monitoring requirements to assess the compliance with the no deterioration objective of the WFD and to assess long term impacts of anthropogenic pressures (Anonymous, 2004). Currently, a guidance document on monitoring in sediment and biota is being drafted under the leadership of France (INERIS, Valeria Dulio), Italy (ISS - Mario Carere and CNR-IRSA – Stefano Polesello) and JRC (Georg Hanke) and under the umbrella of the Chemical Monitoring Activity (CMA) group of the European Commission.

Soil

On 22 September 2006 the EU Commission presented its Thematic Strategy for Soil Protection along with a proposal for a Soil Framework Directive (Com(2006)232) and an Impact Assessment SEC(2006)1165 and SEC(2006)620 for the protection and sustainable use of soil.

The proposal does not mention environmental quality standards and but states that different risk assessment methodologies for contaminated sites are currently being applied in the Member States. The intention is confirmed to come up with a coherent soil protection regime which shall be based on a harmonization of some of the elements of risk assessment. At the same time it is stressed that due to the enormous variability of soil across Europe, a "one-fits-all" approach could probably not be adopted as the basis for Community soil policy. Opinions that reached the EC advised on a flexible system which would allow local specificities of soil and land use to be taken into account. Consequently only if harmonization is needed, the Commission will adopt common criteria for soil contamination risk assessment. In the other cases, it will be left to the Member States to identify contaminated sites and to prepare a “soil status report” if these are sold.

The soil protection scheme in Germany is an example for a relatively complex national assessment as the Federal Soil Protection and Contaminated Sites Ordinance (Bundesbodenschutzverordnung, BBodSchV) differentiates between action, trigger and precautionary values of chemical concentrations in soil. These values differ with regard to different soil uses such as playgrounds, residential areas, parks and recreational facilities, and lands used for industrial and commercial purposes. In order to derive the values toxicological data, pathway-specific exposure, and assessment factors are taken into account (Zeddel & Huhn 1998; http://freenet-homepage.de/zeddel/ARTI_GEF.htm).

---

[3] Expert Advisory Forum on Priority Substances

[4] Expert Group on Analysis and Monitoring of Priority Substances

6.3 Conclusions on metal risk assessment in European legislation

The WFD has been criticized a lot and mostly because some of the EQS chosen are too conservative and overly protective leading to many water bodies failing the compliance check and causing immense costs (Marc Crane, SETAC, presentation 2007).

This, however, is only part of the story, and existent and more adverse problems may be overlooked due to deficiencies in the current regulation.

Using the WFD as an example, the potentials and challenges in today's metals risk assessments are shown and a number of statement concluded that are drawn from this report.

Statement 1: Metal risk assessment on the basis of generalized EQS faces a high uncertainty and may lead to immense and unnecessary costs if they are overly protective, as they do not take bioavailability into account.

Explanation: Variable physico-chemical conditions and species specificities in their impact on bioaccessibility, bioavailability, and bioaccumulation are not taken into account in the derivation of EQS.

Bioavailability of metals in the water has long been recognized as a function of water chemistry. Complex formation and the adsorption of metal ions which depend on pH, temperature, concentration of other ions, and DOC-concentration change the accessibility of metals. An extreme example can be found in the Elbe basin: the Schlenze, a tributary to the Saale, carries highly saline water from former copper mining into the Saale river. While the heavy metals are dissolved in the Schlenze, with decreasing salinity they continuously bind to particles during their transport to the Saale and from there into the Elbe River. At the measuring station at the Elbe in Magdeburg, an elevated salinity is still measurable (Martina Baborowski, UFZ, personal communication, Heise et al. in print). While the metal concentration will be chemically assessed correctly in the total fraction (as long as no SPM settles in the Saale weir and mouth) the effect of these contaminants in different forms on organisms at the sites is unknown so far.

The application of the FIAM indicating the free ion concentration and, more so, of the BLM, that incorporates metal speciation and the protective effects of competing ions for membrane receptors would be more suitable to assess existing risks than general EQS. As has been pointed out before, however, the percentage of diet-borne uptake may be of significant importance and is not covered by these models so far.

In the EQS-determination of the Water Framework Directive, even those suggestions of the FHI-report (Lepper 2005) which would have taken into consideration the background approach (added risk approach) and the reduced bioavailability of particle bound substances (dissolved versus total fraction) were not used.

For soils and sediments, the generation of general EQS is even more complicated, as all gradients of physico-chemical properties are much steeper than in water, and in soils, the influence of acid rain could lead to a profound change in metal bioavailability. In sediments, oxygen gradients occur at the sediment water interface and even across suspended particles. The equilibrium partitioning approach for assessing sediment toxicity has been extended to heavy metals and risk assessment is based on the assumption that the activity of the metals is controlled by the amount of acid-volatile sulphide (AVS; see chapter 4.2). This discounts the fact that bioturbating organisms mostly create their own oxic environment in sediments and may hence be affected by remobilized metal concentrations.

The problem

For some substances, regulation on the basis of Member states has been suggested by the WFD and even more so proposed by the Soil Strategy. While this could lead to a more site-specific and “more correct” approach, science “clashes” here with feasibility. Apart from the immense effort that would need to be put in site-characteristic assessment of the EQS, this could provoke incomparable assessments, and very differently derived and applied quality standards

Alternatives:

In order to counteract the oversimplification of general EQS for metal risk assessment in water, soil and sediments, an approach is needed which is feasible and at the same time addresses the various environmental and physiological influences on bioaccessibility, bioavailability, and bioaccumulation.

a) application of the metalloregion concept

A geographical extension to the metalloregions concept could be applicable. Metalloregions 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 (Janssen *et al.*, 2000). Accordingly, clearly distinguished ranges of natural background concentrations would need to be defined, and PNECs be derived for each of these regions by testing local organisms. Thus, a PNEC derived for a metal-deprived area will not be suitable for a metal-rich area. The metalloregion concept would therewith account for regional parameters affecting metal availability in soil, water and sediment, as well as for the differences in organisms to respond to metal addition (Fairbrother & McLaughlin, 2002).

b) Identifying metal-specific biodynamics

Another possibility, which addresses specifically bioaccumulating processes, is the application of biodynamic models. An example, which has been successfully applied, is the dynamic multi-pathway bioaccumulation model (DYNBAM) (Luoma & Rainbow, 2005). This model considers the uptake of metals via food and therewith responds to one of the major deficiencies identified with the BLM. DYNBAM assumes that net bioaccumulation of metals is the result of a balance among three mechanisms: uptake rate from diet, uptake rate of dissolved forms, and loss rates (Luoma & Rainbow, 2005). DYNBAM and similar models require experimental data measured under environmentally realistic conditions in order to derive model parameters for each metal–species combination (e.g., uptake and elimination rates, assimilation efficiency, food ingestion rates) (Fairbrother et al. 2005).

The advantage of DYNBAM as opposed to static BCF/BAF factors is the consideration of species-specific reactions to ambient concentrations, which again may differ between the kind of metals. While e.g. the abundance of zinc and chromium in the aquatic environment is similar, the uptake of chromium is much lower than zinc. Organisms, that are described as bioaccumulators, hence have low rate constants of loss (Luoma & Rainbow, 2005). Especially good accumulators of zinc e.g. are barnacles. A high rate of ingestion by barnacles together with high Zn assimilation from food, is not matched by the extremely slow rate of zinc excretion until very high concentrations are attained in tissue. The importance of both uptake routs, the uptake via food as well as the uptake via dissolved phase was shown by Luoma (2005) for cadmium: When both routes of uptake were considered, bioaccumulation of cadmium in nature agreed well with the forecasts by DYNBAM. Cadmium bioaccumulation from dissolved sources was within 50% of total Cd bioaccumulation in the bivalves M. edulis and M. balthica from San Francisco Bay.

The question remains, how to use biodynamic models in the development of EQS:

If bioaccumulation is considered one indicator of adverse environmental impact, then species-specific differences have to be elucidated. Some species are likely to be much more vulnerable to metals that others. The aim in the process of EQS identification could be to identify which species are most vulnerable to which metals. Empirical and experimental approaches could yield generalizations about vulnerable species in a reasonable time frame. Internal mechanisms governing uptake (specifically absorption efficiency and loss rates) would also allow generalizations about which metals are likely to be most effectively transferred between trophic levels and for which species. Metals with slow loss rates and high ingestion rates would be strong candidates for trophic transfer. Biomagnification is expected for methyl mercury and selenium, based upon these principles, and could be common for cadmium and zinc[5].

By investigating the different biodynamic processes and species-specific differences, thresholds could be established that protect the vulnerable species from metals.

It has to be stated, though, that bioaccumulation is not equal to toxicity (e.g. (Rainbow, 2002). Due to methods to inactivate the accumulated metals within the organisms, not all substances that have been taken up, are bioactive. Goulet et al. showed for example, that dietary cadmium was not associated with lethal or sub-lethal effects in Daphnia magna, up to very high concentrations, but that waterborne cadmium caused death (Goulet *et al.*, 2007).

Hence, where the risk to aquatic communities needs to be assessed, bioaccumulation in combination with toxicity data need to be established, based on the knowledge of biodynamic and toxicodynamic processes. While the BLM has been shown to predict short-term metal toxicity to various organisms accurately as a function of water quality characteristics, less information is available on the potential use of this concept for assessing metal bioavailability during chronic exposures and/or with other groups of organisms such as unicellular algae (Janssen *et al.*, 2003). Use of BLM for chronic exposure data may, however, become a useful tool to adjust PNEC to the water quality characteristics that are specific for a certain region or for a certain EU wide percentage of surface waters. Research aims at adapting the BLM to chronic exposure assessment and first positive results have been achieved (Schamphelaere *et al.*, 2005). If these models can be further established, the BLM and the DYMBAM may be used to improve the assessment of metal bioavailability, toxicity and bioaccumulation of metals and the development of EQS for sediment which are scientifically sound. An understanding of these processes will offer the possibility to generalize over metals and organisms and establish ecologically justifiable environmental criteria.

---

[5] Samuel N. Luoma and A. Robin Stewart, US Geological Survey, Menlo Park, CA 94025: Processes affecting trophic transfer and resultant effects of metals.

Statement 2: Ignoring sediment in risk assessment schemes could lead to major risks

Explanation:

Even though the main route of exposure is through food and water phase, the benthic community is directly exposed to sediment-bound particles and porewaters. By burrowing and by creating their own environment, benthic organisms may release metals from sediments. Being at the bottom of the food chain, their exposure and bioaccumulation is of high concern. Additionally, sediments may become resuspended during high water discharge which may lead to a remobilization of metals. Up to now, there is a lack of understanding of how much of previously adsorbed metals would become remobilised under environmental conditions and how this is influenced by environmental parameters like temperature, pH, time.

Again an example from the Saale, tributary to the Elbe River. Saale sediments (Peak near Bernburg in 2000) show mercury concentrations of up to 60 mg/kg. At low water discharge, suspended sediments in the Saale mouth can reach concentrations of up to 13 mg/kg when sediments are resuspended probably as a consequence of shipping activity (Heise *et al.*, 2007). The concentrations of Hg in the filtered water samples near Bernburg however were constantly below the environmental quality standard for water (Ms. Kasimier, Sachsen-Anhalt, personal communication). Therewith the contaminated sediment presents a potential risk when resuspended, and an permanent risk to the local benthic community, later also through subsequent transfer in the food chain for pelagic fish and birds. The example from the Saale near Wettin (Figure 6.3) shows the averaged mercury concentrations in muscles of breams between 1995 and 2007, which are far above the proposed EQS-Biota of 20 µg/kg ww.

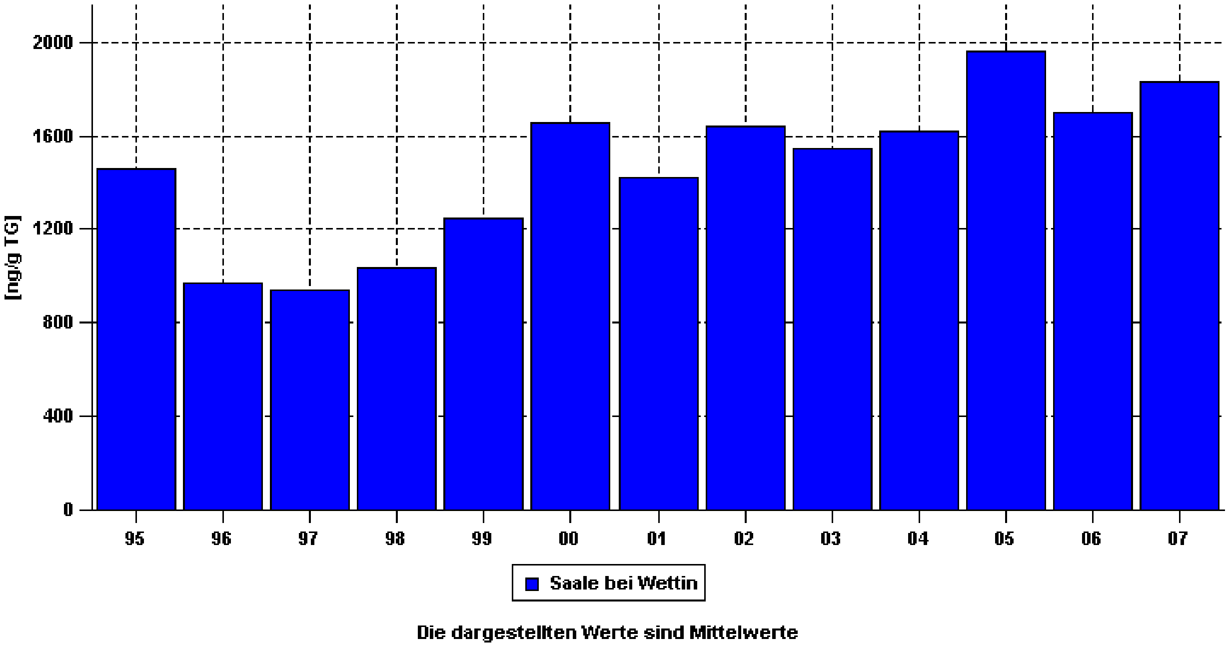


Figure 6.3: Mercury levels in muscle tissue of breams (*Abramis brama*) at the Saale near Wettin. Averaged concentrations for each year are depicted.

The problem:

Mandatory pass/fail, legally enforceable EQS are almost certainly impractical for assessing and managing sediments and biota, as the risk of false positive or negative results is too high (Crane, 2003). Due to the intrinsic heterogeneity and vast diversity of sediments, general EQS will be difficult to establish without them being overly protective.

Alternatives/potential Solutions

a) the application of field studies

In the field, exposure, bioavailability, bioaccumulation and effects are linked, and while linear correlations across complex biological, geochemical or environmental gradients are probably the exception among these processes, statistical analysis of chemical data in the field and measured toxicities could result in conclusions on those levels, below which no adverse affects occur. Crane & Babut suggested such an approach of deriving EQS which places greater emphasis on field data than on extrapolation from laboratory data (Crane & Babut, 2007). From a large data base they selected those sites which showed a good status with regard to benthic macroinvertebrate richness and compared the dissolved nickel concentrations with other sites, assuming that the maximal present nickel concentrations at locations with “good status” could not be inhibiting. They found a 95th percentile of 8.5 µg/L, which is lower than the WFD EQS (20 µg/L) and the national UK standard of 50 to 200 µg/L (depending on water hardness), and higher than the national French (0.5 to 20 µg/L depending on water hardness).

The precondition of applying such an approach is a large database. But while generally applicable, in many cases probably overprotective EQS could be established this concept promises to result in more realistic EQS than the current ones as the example from Crane and Babut showed.

This concept, however, is only applicable for non-mobile sediments and soil. Whether it can also be transferred to suspended matter, which is constantly exposed to changing conditions, is questionable. Also an application to develop EQS for water might be useful for lakes but will not work in rivers, where properties and concentrations of contaminants will be changing constantly.

b) A tiered approach: The development of a tiered approach for sediment risk assessment, making use of multiple lines of evidence (LoI), has been recommended a number of times (den Besten, 2007; Wenning & Ingersoll, 2002) and has been referred to in the discussion on the WFD by Crane and Babut (Crane, 2003). In a weight of evidence-approach, the different LoIs allow for a reduction in uncertainty about the final outcome. According to Wenning and Ingersoll, even though all routes of exposure and types of effects will not occur at every site, “a comprehensive assessment framework should require consideration of the likelihood for all possible routes of exposure and the potential for adverse biological effects to ensure that required or important site-specific environmental factors are not omitted from the evaluation process” (Wenning & Ingersoll, 2002). Again the use of reliable models which do not rely on BCF/BAF-values may facilitate a transparent and – regarding the procedure – harmonized site-specific assessment.

7 Conclusions and Recommendations

Understanding conditions of biomagnification like food web structure and physiological dynamics will allow a greater appreciation of whether ingested metals need to be considered when developing regulatory guidelines designed to protect aquatic animals. It is about time that dietborne metal exposure will be explicitly incorporated into regulatory frameworks even though the links between exposure and effects are not fully understood. However, the bulk metal concentration of a compartment leads to an overestimation of risk not considering bioavaiability of metals, but if accumulation of metals from water and food is additive, assessments of exposure from waterborne metals could underestimate the metal dose to which aquatic animals are exposed.

The equilibrium approach has increased our understanding of some geochemical processes in sediments, but pore water should not be presented as the only available source for organisms living in or on sediments. Alternative methods might be to relate exposure to the metal content in overlaying water, or to make bioavailability corrections, similar to what is proposed for water. The most common regulatory and scientific challenge is the need to determine the ecological implications of sediments contaminated at less than extreme levels. Thus means, pore water, overlaying water, particles in general, and dietborne metals have to be considered as potential uptake routes of metals for benthic organisms. Important uncertainties in the application of the AVS-equilibrium concept exclude this approach for the prediction of bioavailable metal exposure concentrations as either a regulatory tool or a scientific generalization. For a risk assessment, direct measurements of bioavailability and bioaccumulation of metals in living organisms could summarize all influencing factors.

The value of the methods for measuring bioavailability in soils can be significantly improved when the species-, metal-, and soil-specific aspects of bioavailability are more accurately taken into account in the design of chemical simulation methodologies (Peijnenburg et al., 2007). When applying organisms in monitoring programs to estimate the bioavailable concentrations, the relevant uptake route is of minor interest, because the relationship between the metal concentration in the organism and the metal concentration in the sediment or soil can be used for regulatory purposes. However, the link from bioavailable fraction to bioeffective fraction is an important one for a reliable risk assessment. The coherence of a complex environmental system is already considered in monitoring studies. A contaminated area was assessed for Zn, Cr, Cu, Ni, Co and Pb in soils, forage grass, milk from cattle, leafy and non-leafy vegetables. Partitioning pattern of soil revealed high levels of Zn, Cr, and Cu associated with labile fractions, making them more mobile and plant available. The associated risk was assessed using hazard quotient. Human risk was assessed in people known to consume these contaminated foods by analyzing metals concentrations in venous blood and urine (Sridhara Chary et al., 2007).

8 References

Ahlf,W. and Förstner,U. (2001) Managing Contaminated Sediments - I. Improving chemical and biological criteria. JSS **1,** 30-36.

Allen HE and Janssen CR (2006) Incorporating bioavailability into criteria for metals. In Soil and Water Pollution Monitoring, Protection and Remediation. Springer, Netherlands.

Anonymous (2003) Position paper on the derivation of Quality Standards for 'water-total' and the recalculation of monitoring data to standard conditions for Priority Substances in the context of the Water Framework Directive. Draft, version 27-6-2003.

Anonymous (2004) Expert Group on Analysis and Monitoring of Priority Substances (AMPS). Discussion Document, 13 January 2004, 30 p. Ispra. See also: WFD AMPS Sediment Monitoring Guidance Discussion Document, AMPS and SedNet, Draft Version 1 from 16 April 2004.

Ankley, G.T., Di Toro, D.M., Hansen, D.J., Berry, W.J. (1996) Technical basis and proposal for deriving sediment quality criteria for metals. Environ. Toxicol. Chem. 15 (12), 2056–2066.

Batley,G.E., Apte,S.C., and Stauber,J.L. (2004) Speciation and bioavailability of trace metals in water, Progress since 1982. Australian Journal of Chemistry **57,** 903-919.

Berry,W.J., Cantwell,M.G., Edwards,P.A., Serbst,J.R., and Hansen,D.J. (1999) Predicting toxicity of sediments spiked with silver. Environ. Toxicol. Chem. **18,** 40-48.

Blondzik, K., Bunzel, K., Claussen, U., Gluschke, M., Heidemeier, J., Herata, H., et al. (2006) Umweltpolitik. Wasserwirtschaft in Deutschland; Teil 2 – Gewässergüte. - Umweltbundesamt, p. 130.

Borgmann,U., Janssen,C.R., Blust,R., Brix,K.V., Dwyer,R.L., Erickson,R.J. et al. (2005) Incorporation of Dietborne Metals Exposure into Regulatory Frameworks. In Toxicity of Dietborne Metals to Aquatic Organisms. Meyer,J.S., Adams,W.J., Brix,K.V., Luoma,S.N., Mount,D.R., Stubblefield,W.A., and Wood,C.M. (eds). Setac, 153-190.

Calace,N., Petronio,B.M., and Pietroletti,M. (2006) Metal bioavailability, How does its significance change in the time? Annali di Chimica **96,** 131-136.

Calmano,W., Ahlf,W., and Foerstner,U. (1988) Study of Metal Sorption/Desorption Processes on Competing Sediment Components with a Multichamber Device. Environ. Geol. Water Sci. **11,** 77-84.

Crane, M. (2003) Proposed development of Sediment Quality Guidelines under the European Water Framework Directive: a critique. Toxicology Letters 142: 195-206.

Crane, M. and Babut, M. (2007) Environmental Quality Standards for Water Framework Di-rective Priority Substances: Challenges and Opportunities. Integrated Environmental As-sessment and Management: 290-296.

Croteau,M.N., Luoma,S.N., and Stewart,A.R. (2005) Trophic transfer of metals along freshwater food webs, Evidence of cadmium biomagnification in nature. Limnol. Oceanogr. **50,** 1511-1519.

Croteau,M.N., Luoma,S.N., and Pellet,B. (2007) Determining metal assimilation efficiency in aquatic invertebrates using enriched stable metal isotope tracers. Aquatic Toxicology **83,** 116-125.

Croteau,M.N. and Luoma,S.N. (2007) Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand and biodynamics using enriched stable isotopes. Environmental Science & Technology **41,** 3140-3145.

Den Besten, P. Risk assessment approaches in European countries. In: Heise, S. (ed.) Sedi-ment Risk Management and Communication. Elsevier, 2007

De Schamphelaere,K.A.C., Canli,M., Van Lierde,V., Forrez,I., Vanhaecke,F., and Janssen,C.R. (2004) Reproductive toxicity of dietary zinc to Daphnia magna. Aquatic Toxicology **70,** 233-244.

De Schamphelaere,K.A.C. and Janssen,C.R. (2004) Development and field validation of a biotic ligand model predicting chronic copper toxicity to Daphnia magna. Environ. Toxicol. Chem. **23,** 1365-1375.

De Schamphelaere,K.A.C. and Janssen,C.R. (2004) Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to Daphnia magna. Environ. Toxicol. Chem. **23,** 1115-1122.

De Schamphelaere, K. A. C., Lofts, S. and Janssen, C. R. (2005) Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters. Environmental Toxicology and Chemistry 24: 1190–1197.

De Vries,W., Lofts,S., Tipping,E., Meili,M., Groenenberg,J.E., and Schutze,G. (2007) Impact of soil properties on critical concentrations of cadmium, lead, copper, zinc, and mercury in soil and soil solution in view of ecotoxicological effects. Reviews of Environmental Contamination and Toxicology, Vol 191 **191,** 47-89.

Deleebeeck,N.M.E., Muyssen,B.T.A., De Laender,F., Janssen,C.R., and De Schamphelaere, K.A.C. (2007) Comparison of nickel toxicity to cladocerans in soft versus hard surface waters. Aquatic Toxicology **84,** 223-235.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, and Paquin PR (2001) Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. **20,** 2383-2396.

Di Toro,D.M., McGrath,J.A., Hansen,D.J., Berry,W.J., Paquin,P.R., Mathew,R. et al. (2005) Predicting sediment metal toxicity using a sediment biotic ligand model, Methodology and initial application. Environ. Toxicol. Chem. **24,** 2410-2427.

EC (2000): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities L327/1, 22 December 2000

EC (2003): Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances. European Commission. Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. 2nd Edition. Ispra, Italy,

Ehlers,L.J. and Luthy,R.G. (2003) Contaminant bioavailability in soil and sediment. Environmental Science & Technology **37,** 295A-302A.

EPA (2007): Framework for Metals Risk Assessment. United States Environmental Protection Agency. EPA 120/R-07/001. Washington, DC, March 2007. 172

Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson Jr., H.P., Leonard, E.N., 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem. 15 (2), 181–193.

Escher,B.I. and Hermens,J.L. (2004) Internal exposure, linking bioavailability to effects. Environ. Sci. Technol. **38,** 455A-462A.

Fairbrother, A. and Mclaughlin, M. (2002) Fact Sheet on Environmental risk assessment 12: Metalloregions. - International Council on Mining and Metals (ICMM)

Fairbrother,A., Wenstel,R., Sappington,K., and Wood,W. (2007) Framework for metals risk assessment. Ecotoxicology and Environmental Safety **68,** 145-227.

Frische, T., Mebes, K-H., Filser, J. (2003) Assessing the bioavailability of contaminants in soils: A review on recent concepts. UBA report 66/03, 151 p.

Goulet, R. R., Krack, S., Doyle, P. J., Hare, L., Vigneault, B. and Mcgeerd, J. C. (2007) Dy-namic multipathway modeling of Cd bioaccumulation in Daphnia magna using waterborne and dietborne exposures. Aquatic Toxicology 81: 117-125.

Griscom,S.B., Fisher,N.S., Aller,R.C., and Lee,B.G. (2002) Effects of gut chemistry in marine bivalves on the assimilation of metals from ingested sediment particles. Journal of Marine Research **60,** 101-120.

Guo,L.D., Santschi,P.H., and Ray,S.M. (2002) Metal partitioning between colloidal and dissolved phases and its relation with bioavailability to American oysters. Marine Environmental Research **54,** 49-64.

Hare,L., Tessier,A., and Warren,L. (2001) Cadmium accumulation by invertebrates living at the sediment-water interface. Environ. Toxicol. Chem. **20,** 880-889.

Hassler,C.S., Slaveykova,V.I., and Wilkinson,K.J. (2004) Some fundamental (and often overlooked) considerations underlying the free ion activity and biotic ligand models. Environ. Toxicol. Chem. **23,** 283-291.

Heise, S., Krüger, F., Baborowski, M., Stachel, B., Götz, R. and Förstner, U. (2007) Bewer-tung der Risiken durch Feststoffgebundene Schadstoffe im Elbeeinzugsgebiet. - Im Auf-trag der Flussgebietsgemeinschaft Elbe und Hamburg Port Authority, erstellt vom Be-ratungszentrum für integriertes Sedimentmanagement (BIS/TuTech) an der TU Hamburg-Harburg, p. 349.

Hsu,P., Matthäi,A., Heise,S., and Ahlf,W. (2007) Seasonal Variation of Sediment Toxicity in River Basins of Dommel and Elbe. Environmental Pollution **148,** 817-823.

ICMM (2007): MERAG: Metals Environmental Risk Assessment Guidance. International Council on Mining and Metals. London, UK, 84

Janssen, C. R., De Schamphelaere, K., Heijerick, D., Muyssen, B., Lock, K., Bossuyt, B., et al. (2000) Uncertainties in the environmental risk assessment of metals. Humans and Ecological Risk Assessment 6: 1003-1018.

Janssen, C. R., Heijerick, D. G., De Schamphelaere, K. A. C. and Allen, H. E. (2003) Environmental risk assessment of metals: tools for incorporating bioavailability. Environment International 28: 793– 800.

Kamunde,C., Clayton,C., and Wood,C.M. (2002) Waterborne vs. dietary copper uptake in rainbow trout and the effects of previous waterborne copper exposure. American Journal of Physiology-Regulatory Integrative and Comparative Physiology **283,** R69-R78.

Lee,B.G., Lee,J.S., Luoma,S.N., Choi,H.J., and Koh,C.H. (2000) Influence of acid volatile sulfide and metal concentrations on metal bioavailability to marine invertebrates in contaminated sediments. Environmental Science & Technology **34,** 4517-4523.

Lepper P (2005): Manual on the Methodological Framework to Derive Environmental Quality Standards for Priority Substances in accordance with Article 16 of the Water Framework Directive (2000/60/EC). Fraunhofer-Institute Molecular Biology and Applied Ecology. Schmallenberg, 15 September 2005. 51

Liss,W. and Ahlf,W. (1997) Evidence from whole sediment, pore water, and elutriate testing in toxicity assessment of contaminated sediments. Ecotoxicol. Environ. Saf. **36,** 140-147.

Liu,J.C., Yan,C.L., Macnair,M.R., Hu,J., and Li,Y.H. (2007) Vertical distribution of acid-volatile sulfide and simultaneously extracted metals in mangrove sediments from the Jiulong River Estuary, Fujian, China. Environmental Science and Pollution Research **14,** 345-349.

Lock,K., Van Eeckhout,H., De Schamphelaere,K.A.C., Criel,P., and Janssen,C.R. (2007) Development of a biotic ligand model (BLM) predicting nickel toxicity to barley (Hordeum vulgare). Chemosphere **66,** 1346-1352.

Lorenzo,J.I., Beiras,R., Mubiana,V.K., and Blust,R. (2005) Copper uptake by Mytilus edulis in the presence of humic acids. Environ. Toxicol. Chem. **24,** 973-980.

Lu,X.Q., Werner,I., and Young,T.M. (2005) Geochemistry and bioavailability of metals in sediments from northern San Francisco Bay. Environment International **31,** 593-602.

Luoma,S.N. and Rainbow,P.S. (2005) Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. **39,** 1921-1931.

Oomen,A.G., Mayer,P., , Tolls,J. (2000) Nonequilibrium solid phase microextraction for determination of the freely dissolved concentration of hydrophohic organic compounds: Matrix effects and limitations. *Analytical Chemistry* **72,** 2802-2808.

Ma, H., Kim, S.D., Cha, D.K., Allen, H.E., 1999. Effect of kinetics of complexation by humic acid on toxicity of copper to Ceriodaphnia dubia. Environ. Toxicol. Chem. 18 (5), 828–837.

Martin,A.J. and Goldblatt,R. (2007) Speciation, Behavior, and Bioavailability of Copper Downstream of a Mine-Impacted Lake. Environ. Toxicol. Chem. **26** (12) online first

McGeer, J.C., Szebedinszky, C., McDonald, D.G., Wood, C.M. (2002) The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure. Comp. Biochem. Phys. 133, 147–160.

Mertens,J., Degryse,F., Springael,D., and Smolders,E. (2007) Zinc toxicity to nitrification in soil and soilless culture can be predicted with the same biotic ligand model. Environmental Science & Technology **41,** 2992-2997.

Meyer,J.S. (2002) The utility of the terms "bioavailability" and "bioavailable fraction" for metals. Marine Environmental Research **53,** 417-423.

MERAG (2007) Incorporation of Bioavailability for water, Soils and Sediments. Metals Risk Assessment Guidance (MERAG) Fact Sheet 05. ICMM, London, 23 pp. Published on-line

Nahmani,J., Hodson,M.E., and Black,S. (2007) A review of studies performed to assess metal uptake by earthworms. Environmental Pollution **145,** 402-424.

Ortiz,O. and Alcaniz,J.M. (2006) Bioaccumulation of heavy metals in *Dactylis glomerata* L. growing in a calcareous soil amended with sewage sludge. Bioresour. Technol. **97,** 545-552.

Peijnenburg,W.J.G.M. and Jager,T. (2003) Monitoring approaches to assess bioaccessibility and bioavailability of metals, Matrix issues. Ecotoxicology and Environmental Safety **56,** 63-77.

Peijnenburg,W.J.G.M., Zablotskaja,M., and Vijver,M.G. (2007) Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. Ecotoxicology and Environmental Safety **67,** 163-179.

Rainbow,P.S. and Wang,W.X. (2001) Comparative assimilation of Cd, Cr, Se, and Zn by the barnacle Elminius modestus from phytoplankton and zooplankton diets. Marine Ecology-Progress Series **218**, 239-248.

Rainbow PS. 2007. Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environ Int* 33:576-582.

Reiley,M.C. (2007) Science, polic, and trends of metals risk assessment at EPA, How understanding metals bioavailability has changed metals risk assessment at US EPA. Aquatic Toxicology **84,** 292-298.

Roditi,H.A., Fisher,N.S., and Sanudo-Wilhelmy,S.A. (2000) Field testing a metal bioaccumulation model for zebra mussels. Environmental Science & Technology **34,** 2817-2825.

Sanchez-Marin,P., Lorenzo,J.I., Blust,R., and Beiras,R. (2007) Humic acids increase dissolved lead Bioavailability for marine invertebrates. Environmental Science & Technology **41,** 5679-5684.

Santore,R.C., Di Toro,D.M., Paquin,P.R., Allen,H.E., and Meyer,J.S. (2001) Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. Environ. Toxicol. Chem. **20,** 2397-2402.

Schlekat, C.E., Luoma, S.N., 2000. You are what you eat, Incorporating dietary metals uptake into environmental quality guidelines for aquatic ecosystems, SETAC Globe, v. 1(2), p. 38-39.

Scott-Fordsmand,J.J., Weeks,J.M., and Hopkin,S.P. (2000a) Importance of contamination history for understanding toxicity of copper to earthworm Eisenia fetica (Oligochaeta , Annelida), using neutral-red retention assay. Environ. Toxicol. Chem. **19,** 1774-1780.

Scott-Fordsmand,J.J., Krogh,P.H., and Weeks,J.M. (2000b) Responses of Folsomia fimetaria (Collembola , Isotomidae) to copper under different soil copper contamination histories in relation to risk assessment. Environ. Toxicol. Chem. **19,** 1297-1303.

Scott-Fordsmand,J.J., Stevens,D., and McLaughlin,M. (2004) Do earthworms mobilize fixed zinc from ingested soil? Environmental Science & Technology **38,** 3036-3039.

Simpson,S.L. and King,C.K. (2005) Exposure-pathway models explain causality in whole-sediment toxicity tests. Environmental Science & Technology **39,** 837-843.

Sofyan,A.S.J.R.B.W.J. (2006) Metal Trophic Transfer from Algae to Cladocerans and the Relative importance of Dietary metal Exposure. Environ. Toxicol. Chem. **25,** 1034-1041.

Sridhara Chary,N., Kamala,C.T., and Samuel Suman Raj,D. (2007) Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicology and Environmental Safety **In Press, Corrected Proof,** 647.

Steenbergen,N.T.T.M., Iaccino,F., De Winkel,M., Reijnders,L., and Peijnenburg,W.J.G.M. (2005) Development of a biotic ligand model and a regression model predicting acute copper toxicity to the earthworm Aporrectodea caliginosa. Environmental Science & Technology **39,** 5694-5702.

Strandberg,B., Axelsen,J.A., Pedersen,M.B., Jensen,J., and Attrill,M.J. (2006) Effect of a copper gradient on plant community structure. Environ. Toxicol. Chem. **25,** 743-753.

Sundelin,B. and Eriksson,A.K. (2001) Mobility and bioavailability of trace metals in sulfidic coastal sediments. Environ. Toxicol. Chem. **20,** 748-756.

Thakali,S., Allen,H.E., Di Toro,D.M., Ponizovsky,A.A., Rooney,C.P., Zhao,F.J., and McGrath,S.P. (2006) A Terrestrial Biotic Ligand Model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. Environmental Science & Technology **40,** 7085-7093.

Thakali,S., Allen,H.E., Di Toro,D.M., Ponizovsky,A.A., Rooney,C.P., Zhao,F.J. et al. (2006) Terrestrial biotic ligand model. 2. Application to Ni and Cu toxicities to plants, invertebrates, and microbes in soil. Environmental Science & Technology **40,** 7094-7100.

Twining,B.S. and Fisher,N.S. (2004) Trophic transfer of trace metals from protozoa to mesozooplankton. Limnol. Oceanogr. **49,** 28-39.

US EPA (Environmental Protection Agency) (2003) Guidance for the Development of Ecological Soil Screening Levels. Office of Solid Waste and Emergency Response, Washington, DC (OSWER Directive 92857-55).

Van Leeuwen,H.P. and Pinheiro,J.P. (2001) Speciation dynamics and bioavailability of metals. Exploration of the case of two uptake routes. Pure and Applied Chemistry **73,** 39-44.

Vijver,M.G., Van Gestel,C.A.M., Lanno,R.P., Van Straalen,N.M., and Peijnenburg,W.J.G.M. (2004) Internal metal sequestration and its ecotoxicological relevance, A review. Environmental Science & Technology **38,** 4705-4712.

Vijver,M.G., Van Gestel,C.A.M., Van Straalen,N.M., Lanno,R.P., and Peijnenburg,W.J.G.M. (2006) Biological significance of metals partitioned to subcellular fractions within earthworms (Aporrectodea caliginosa). Environ. Toxicol. Chem. **25,** 807-814.

Walker LA, Bailey LJ, and Shore RF (2002) The importance of the gut and its contents in prey as a source of cadmium to predators. Environ. Toxicol. Chem. **21,** 76-80.

Wang,W.X. and Ke,C. (2002) Dominance of dietary intake of cadmium and zinc by two marine predatory gastropods. Aquat. Toxicol. **56,** 153-165.

Wenning RJ, Ingersoll CG (2002): Summary of the SETAC Pellston Workshop on Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments. Society of Environmental Toxicology and Chemistry (SETAC). Pensacola FL, USA. Fairmont, Montana, USA, 17-22 August 2002.

Worms,I., Simon,D.F., Hassler,C.S., and Wilkinson,K.J. (2006) Bioavailability of trace metals to aquatic microorganisms, importance of chemical, biological and physical processes on biouptake. Biochimie 88, 1721-1731.

Xu Y and Wang W-X (2002) Exposure and potential food chain transfer factor of Cd, Se, and Zn in marine fish *Lutjanus argentimaculatus*. Marine Ecology Progress Series 238, 173-186.

9 List of Abbreviations

AF	Assessment factor
AVS	Acid volatile sulfides
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BLM	Biotic ligand model
CEC	Cation exchange capacity
CSTEE	Scientific Committee on Toxicity, Ecotoxicity, and the Environment
DOC	Dissolved organic carbon
DTPA	Diethylene diamine pentaacetic acid
DYNBAM	Dynamic multi-pathway bioaccumulation model
EPA	United States Environmental Protection Agency
EqP	Equilibrium partitioning
EQS	Environmental quality standard
FHI	Fraunhofer Institut
FIAM	Free ion activity model
MAC	Maximum accecptable concentration
MERAG	Metals environmental risk assessment guidance
MINTEQ	Geochemical model
MPA	Maximum permissible addition
NOEC	No-observed-effect concentration
OM	Organic material
PNEC	Predicted no effect concentration
PHS	Priority hazardous substance
PS	Priority substance
RAR	Risk assessment report
REACH	Registration, Evaluation and Authorization of Chemicals
SCAMP	Southern California Areal Mapping Project
SEC	Securities and Exchange Commission
SEM	Simultaneously extracted metals
SPM	Suspended particulate matter
SSD	Species sensitivity distribution
TBLM	Terrestrial biotic ligand model
TGD	Technical Guidance Document
WFD	Water Framework Directive
WHAM	Windermere humic aqueous model