Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/22695 holds various files of this Leiden University dissertation

Author: Verschoor, Aletta Johanna
Title: The power of biotic ligand models : site-specific impact of metals on aquatic communities
Issue Date: 2013-12-04

Chapter 1

Introduction: Metals in the aquatic environment

Sources

Metals are natural elements in the environment, captured in rocks and minerals (Salminen, 2006). Through weathering they are dissolved at low concentrations in water, thus becoming a natural trace element in surface and groundwater (Stumm et al., 1996). In modern times - also referred to as the anthropocene by Nobel prize winner P. Crutzen (Crutzen et al., 2000) – the release of metals into the environment has accelerated by human activities such as mining, use of metal-containing products, spills and waste. On top of natural background concentrations, anthropogenic emissions cause an enrichment, which is currently estimated to be in the same order of magnitude as the natural sources, weathering and volcanic activity (UNEP, 2013). As a consequence, biologically available metal concentrations have risen to levels that organisms have not been adapted to. The most important anthropogenic sources of metal emissions world-wide are mining and ore processing, artisanal gold-mining, lead smelting, lead-acid battery recycling, chemical manufacturing, dye industries and tannery operations, and industrial and municipal dumpsites (Blacksmith Institute, 2012).

Metal emissions are increasingly caused by non-metal sources, especially fossil fuels and phosphate rock because 1) environmental regulations have been enforced to reduce the traditional point source emissions in most developed countries and 2) because there is a growing demand of fossil fuel and phosphates (fertilizers) (UNEP, 2013). Besides point sources, there are many diffuse sources

that continue to release metals in the environment, and cause "a blanket" of contamination covering large areas and waterbodies. Examples of diffuse sources of metal emissions to surface water are agriculture, traffic, urban waste water discharges, contaminated soils and sediments, and building materials and recycled waste (i.e. sewage sludge, incinerator slags) in outdoor constructions. Improvement of the environmental quality by reduction of diffuse sources is obstructed by the scattered origin of diffuse emissions and large areas involved.

Because of the natural co-occurrence of metals in minerals and rocks, emissions usually involve a cocktail of metals. For example, Zn-sulfide ore, contains residues of Ag, Au, Ga, Ge, Cd, Co, As and Bi which can be processed as a by-product, but also residues of Mg, Mn, Fe, Cu, Hg, Sb and Tl, that will and up in waste streams (UNEP, 2013).

Effects

Many metals fulfill essential metabolic functions in organisms. Living organisms require optimal internal concentrations of metals, like Cr, Co, Cu, Fe, Mn, Mo, Ni, Se and Zn. Only of As, Cd, Hg and Pb, no beneficial use is known (Peijnenburg et al., 2007).

There is a broad concentration range for optimal biological performance; the so-called "window of essentiality" (Hopkin, 1989). Because the variation in natural metal concentrations is high, organisms have developed a mechanism to actively take up, store and excrete metals from the environment, in order to maintain optimal internal metal concentrations. Concentrations below the window of essentiality cause deficiency symptoms, whereas metal concentrations above the physiological boundaries may lead to accumulation, and eventually to toxic effects.

Metals disturb the uptake of Na and Ca, which are important ions involved in energy metabolism and osmoregulation. Na uptake from the water across the gills is essential for any water breathing freshwater animal, because the diffusive loss of Na from its concentrated extracellular fluids to the surrounding dilute environment has to be compensated (Grosell et al., 2002). Cu and Ag ions in freshwater fish disturb Na homeostasis by inhibition of the enzyme Na-K-ATPase. This enzyme is primarily found in cells of the fish gills and the iono-regulatory epithelia of other aquatic organisms (Paquin et al., 2002b). Other metals were shown to affect Ca uptake and regulation: Co (De Schamphelaere et al., 2008), and Pb (Grosell et al., 2009) in the snail *Lymnea stignalis,* Zn in *Daphnia magna*

(Muyssen et al., 2006), and Cd in the fish *Oncorhynchus mykiss* (Verbost et al., 1989).

Metals are required for highly specific enzymatic reactions, but this also explains their mode of toxic action. Metals are generally involved in oxidation state reactions and alkylation-dealkylation reactions (Finney et al., 2003). Disturbance in enzymatic processes, actually results in visible deformations in individual species and in changes in populations and communities. Abnormal larvae and skeleton malformations were observed when sea urchin was exposed to a Zn-concentration just above the EQS (Carballeira et al., 2012). Deformations on the mouth part of the midge *Chironomus* were induced by Cu and Pb at sublethal concentrations ranges, i.e. the number of teeth were reduced and the mouth gap was smaller (Janssens de Bisthoven et al., 1998). In fish, a variety of effects was observed after exposure to Cd, Cu and Pb: swollen eggs, and larvae with swollen heart, deformed spine, incomplete eye pigmentation and larvae without head or without tail (Jezierska et al., 2009). It is obvious that these deformations will negatively impact the survival of the species in an ecosystem, because they become more susceptible for predators, less successful in obtaining food and less successful in reproduction.

Changes to the community structure due to metal exposure was observed in aquatic mesocosms (Bere et al., 2012) and field studies (Beltman et al., 1999; Courtney et al., 2002; Doi et al., 2007; Fukunaga et al., 2011). Lower number of taxa, loss of metal-sensitive species (ephemeropteran species such as mayflies and stoneflies) and lower total biomass were observed in metal-contaminated surface waters. Although most aquatic organisms possess mechanisms to detoxify or eliminate metals, these processes will proceed at the expense of other energy-demanding processes such as mobility, growth and reproduction and ultimately to reduced survival of species. Thus, also at sub-lethal concentration levels, sensitive species may disappear from the ecosystem and tolerant species will eventually become dominant. Chemical pollution is one of the causes of decreasing biodiversity and loss of ecosystem functions (Millennium Ecosystem Assessment, 2005).

Environmental quality criteria (EQS)

EQS are legally set concentration limits that represent a certain level of protection to the aquatic ecosystem. The predicted no-effect level (PNEC) of a substance is generally adopted as EQS. The PNEC is usually derived from observed no-effects concentrations (NOEC) in toxicity tests. Risks are estimated by comparison of the predicted (or measured) environmental concentration (PEC) with the PNEC.

EQS are values that enable the classification of sites that are "at risk" ($PEC/PNEC > 1$) and sites that are "not at risk" ($PEC/PNEC < 1$). These EQS assist risk managers and regulators to select and prioritize sites that need intervention, to decide if certain activities (for example industrial discharges) can be permitted or to decide if risk mitigation measures should be taken. Derivation and implementation of EQS started about 40 years ago. The EQS were updated several times to reflect the latest insights in science and society. For instance, dissolved metal concentrations replaced total concentrations as EQS in surface water (EC, 2008) and an increasing number of tested species enabled the introduction of species sensitivity distributions which reduced safety factors (EC, 2011) in the environmental quality standards.

Currently, EQS for most metals are expressed as dissolved concentrations. The EQS refers to metal concentrations after filtration over 0.45 µm and excludes metals that are bound to suspended matter. Although dissolved concentration better explained the variation in toxic effects, differences in toxicity were still observed in surface waters with equal dissolved metal concentrations. This variation was explained by differences in the biological availability of dissolved metals. Bioavailability is a term that was first used since 1969 in scientific literature (Allen, 1969) in relation to efficacy of pharmaceutical drugs. The first traceable literature were bioavailability is used in an environmental context is published in 1976 (Patrick Wm Jr. et al., 1977). Bioavailability was adopted by environmental scientists to describe the phenomenon that only a particular part of total metal present has a biological effect. A molecule is said to be bioavailable when it is available to cross an organism's cellular membrane from the environment (Semple et al., 2004).

The effect of bioavailability on metal toxicity is legally acknowledged for Cd. Because Cd effects were strongly related to hardness (Carroll et al., 1979; Verbost et al., 1989) five hardness classes have been distinguished with corresponding EQS (EC, 2008). Except for Cd, the EQS are equal for all freshwater systems,

regardless of their specific water chemistry, and are referred to as generic EQS. For all other metals it is recognized that it is important to include bioavailability in the environmental risk assessment. In footnotes of European Directive 2008/105/EC it is stated that: "Member States may, when assessing the monitoring results against the EQS, take into account: hardness, pH or other water quality parameters that affect the bioavailability of metals." No recommendations are given on how or what model should be used.

Bioavailability

Bioavailability became an issue in aquatic ecotoxicology because it was observed that toxic effects in laboratory tests were often higher than observed in field samples with equal metal concentrations (Allen et al., 1996). It appeared that the free ionic form of a metal was mainly responsible for the toxic effect (Sunda et al., 1978; Vink, 2002; 2009; Zamuda et al., 1982), and that natural water samples contain constituents that reduce the free ion activity of metals.

Dissolved organic carbon, hardness and pH were the first parameters found to be largely responsible for modification of metal toxicity (Pagenkopf, 1983; Zitko et al., 1976). The role of dissolved organic matter as component that reduces toxicity is based on its capacity to form metal-complexes and thus reduce free metal ion activities (Bryan et al., 2002; De Schamphelaere et al., 2004a; Doig et al., 2006; Slaveykova, 2007).

Several methods exist to measure free ion activities, such as ion selective electrodes, diffusive gradients in thin-film gels (DGT), gel integrated microelectrode arrays (GIME) combined with voltammetric measurements; stripping chrono-potentiometry (SCP); flow-through and hollow fiber permeation liquid membranes (FTPLM and HFPLM); Donnan membrane technique (DMT), competitive ligand-exchange stripping voltammetry and adsorption stripping voltammetry (Sigg et al., 2006). The speciation in undisturbed sediment-water systems can be measured by SOFIE (Sediment Or Fauna Incubation Experiment) in combination with micro ion-exchange columns (MIC)(Vink et al., 2005). These methods are less suitable for regulatory monitoring purposes, and therefore monitoring databases usually contain concentrations of total (dissolved) metals.
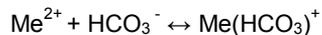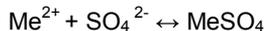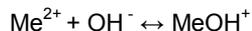
Because free ion activities are not routinely measured in surveillance monitoring programs, corrections were necessary to improve the risk assessment methodology. As a first step bioavailability was approached by excluding the metals bound to suspended matter from the risk assessment, either by filtering

water samples prior to analysis, or by posterior mathematical correction for binding to suspended matter (Garnier et al., 1997; Sauvé et al., 2000).

Currently the filtering of water samples prior to analysis is common practice and generic EQS are expressed as such. When a refined risk assessment is desired, free ion activities can be computed from dissolved concentrations with speciation models. When the total dissolved concentrations of metals and other elements are known, free ion activities can be computed by speciation models. Speciation reactions are implemented in software programs of which WHAM (Tipping, 1994; 1998; Tipping et al., 2011), and MINEQL (www. mineql.com) are most commonly used to calculate speciation in aquatic systems.
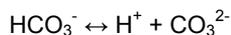
The chemical reactions of metals with DOC and inorganic ligands are described by equilibrium reactions (in which Me^{2+} is a metal ion, for example, Zn^{2+}, Cu^{2+}, Ni^{2+} etcetera):

<div style="border:1px solid black; padding:1em;">

Metal complexation

$$Me^{2+} + DOC \leftrightarrow Me\text{-}DOC$$

$$Me^{2+} + OH^- \leftrightarrow MeOH^+$$

$$Me^{2+} + SO_4^{2-} \leftrightarrow MeSO_4$$

$$Me^{2+} + Cl^- \leftrightarrow MeCl^+$$

$$Me^{2+} + HCO_3^- \leftrightarrow Me(HCO_3)^+$$

</div>

The carbonate system has a large impact on chemical equilibria because it interacts with the pH and with the metal.

<div style="border:1px solid black; padding:1em;">

Carbonate system:

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$$

</div>

Chemical equilibria in the aquatic system are described by K-values. The K-value determines the relative amounts of different chemical species in a water sample. For example, the reaction of a metal with bicarbonates is described by:

$$K = \frac{[MeHCO_3^+]}{[Me^{2+}] \times [HCO_3^-]}$$

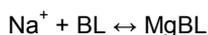in which concentrations are expressed in mol/L.

High K-values indicate that the equilibrium tends to shift to the right, i.e. complexation is relatively high. The distribution of a metal over different chemical species depends of the water chemistry, i.e. on the total concentrations of metal, pH, DOC and inorganic ligands.

To predict toxic effects or PNEC of free metal ions, models were developed that account for the actual binding of active metal-species to biological surfaces. The Gill Surface Interaction Model was the first model to combine the effects of Ca^{2+} with the effects of metal complexation (Pagenkopf, 1983). This concept is further elaborated in the biotic ligand model (Di Toro et al., 2001; Paquin et al., 2002a).

Biotic ligand model

Biotic ligand models (BLMs) are the state-of-the-art approach to account for the effect of bioavailability on metal toxicity. The aim of BLMs is to derive a water type-specific effect concentration (NOEC, EC_{50}). BLMs combine two phenomena which affect metal toxicity (see Figure 1.1): 1) chemical speciation, that determines the concentration of free ion activity of the metal and other ions in water and, 2) competitive binding at the biotic ligand, also referred to as the hardness effect.

<div style="border:1px solid black">

<u>Hardness effect:</u>

$Ca^{2+} + BL \leftrightarrow CaBL$

$Mg^{2+} + BL \leftrightarrow MgBL$

$Na^+ + BL \leftrightarrow MgBL$

$H^+ + BL \leftrightarrow HBL$

$Me^{2+} + BL \leftrightarrow MeBL$

</div>

14

BLMs elaborate on the observation that free metal ions interfere with the uptake of Ca, Mg and Na and vice versa, the so-called hardness effect. Similar to inorganic ligand binding as described in the previous paragraph, biotic ligand binding is described by an equilibrium reaction. The affinity of cations for the biotic ligand is described by a K-value. Higher K-values imply a higher affinity of the metal for the biotic ligand. Except for fish, where K-values were directly derived from measured metal-binding to the fish gill, K-values are empirical values, derived during BLM parameterization (De Schamphelaere et al., 2002b).
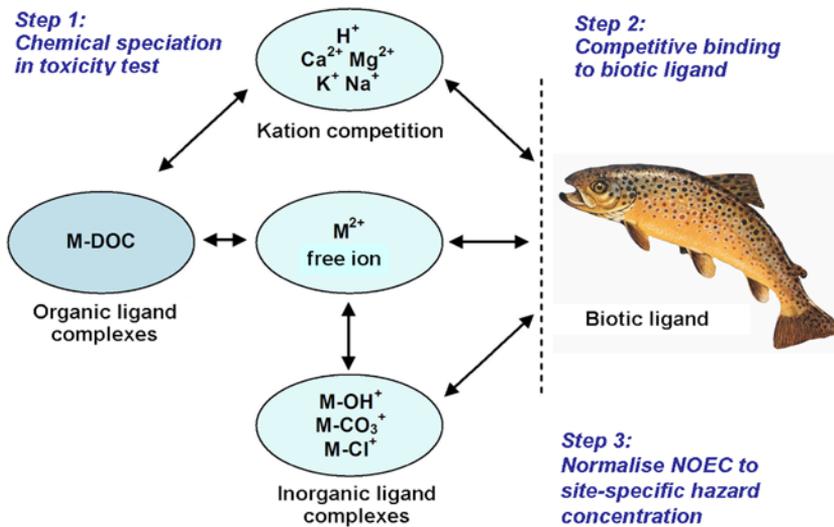

Figure 1.1 Schematic of biotic ligand binding

To calculate a water type-specific effect concentration, BLMs calculate the binding of the metal to the biotic ligand at the effect concentrations found in standard laboratory toxicity tests ($f_{BL,x\%}$). Analogue to EC_x, $f_{BL,x\%}$ is a measure for the intrinsic sensitivity of a species, and it is derived from toxicity tests. The $f_{BL,x\%}$ represents the fractional metal occupancy of the biotic ligand where x% of a population will be affected:

$$f_{BL,x\%} = \frac{K_{Me-BL} \times \alpha_{Me}}{1 + K_{Me-BL} \times \alpha_{Me} + K_{Ca-BL} \times \alpha_{Ca} + K_{Mg-BL} \times \alpha_{Mg} + K_{Na-BL} \times \alpha_{Na} + K_{H-BL} \times \alpha_{H}}$$

in which K= affinity constant for biotic ligand binding and α_{Me} = EC_x expressed as free metal ion activity. For the calculation of EC_x in natural water samples it is computed at what free metal activity (α_{Me}) the occupancy of the biotic ligand (f_{BL})

equals $f_{BL,50\%}$, at the given amounts of Ca, Na and Mg in that surface water sample:

$$EC_x = \frac{f_{BL,x\%}}{1-f_{BL,x\%}} \times \left\{ 1+K_{Ca-BL} \times \alpha_{Ca}+K_{MgBL} \times \alpha_{Mg}+K_{Na-BL} \times \alpha_{Na}+K_{H-BL} \times \alpha_H \right\}$$

BLMs were generally able to explain differences in toxicity between sites and estimate toxic effects within a factor of 2 accuracy (Bielmeyer et al., 2007; De Schamphelaere et al., 2002a; De Schamphelaere et al., 2003; De Schamphelaere et al., 2004c; Deleebeeck et al.; Heijerick et al., 2002b; Lock et al., 2006; Schwartz et al., 2007; Villavicencio et al., 2011).

Starting in 1999, the number of publications until end of 2012 amounted 367 (Scopus, keyword "biotic ligand model"). After a gradual increase of publications about BLMs in the period 1999-2003, the number of publications is more or less stable at 30 to 38 publications per year. By far the most studies have been performed to Cu, followed by Cd, Zn, Ni and Ag.
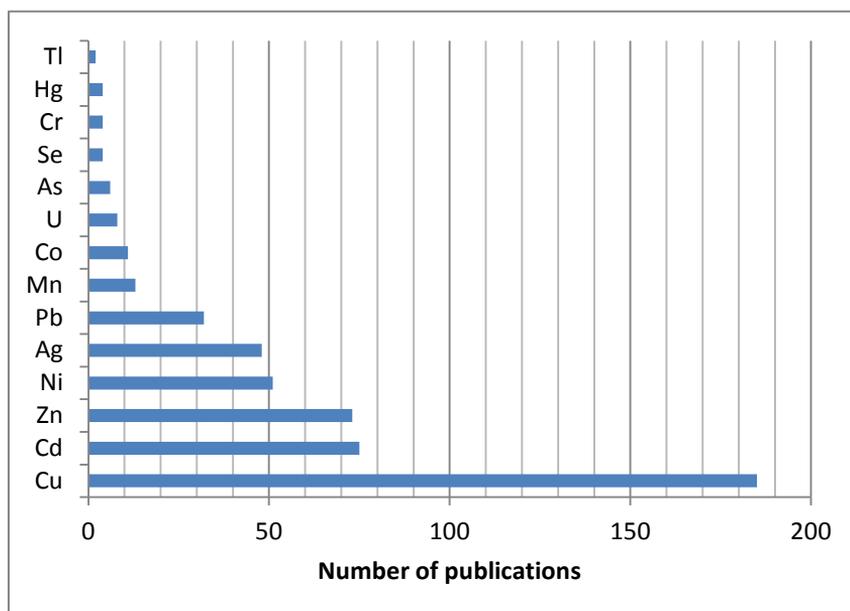

Figure 1.2 Number of publications for each metal, related to biotic ligand models. In Scopus (accessed: 29-4-2013) the full metal name was searched in combination with the keyword "biotic ligand model".

Biotic ligand model development requires extensive laboratory testing. Repeated dose-response tests, at different pH, Ca, Na and Mg and metal concentrations were performed to derive the K-values and $f_{BL,50\%}$. Tests for the aquatic

environment were developed for representatives of several taxonomic groups: fish (*Pimephales promelas* and *Oncorhynchus mykiss*), crustaceans (*Ceriodaphnia dubia* and *Daphnia magna*), and algae (*Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardii*). BLMs were shown to be valid for other species too (De Schamphelaere et al., 2006; De Schamphelaere et al., 2010; Esbaugh et al., 2012; Schlekat et al., 2010). In this way, BLMs were combined with species sensitivity distributions to determine water type-specific PNECs.

Although the concept was accepted by the European Water Framework Directive, and BLMs were available for several metals and species, the practical implementation stayed behind because of model complexity, data requirement, uncertainty about the validity for field predictions and consequences of BLMs for risk assessment.

Scope and purpose of PhD thesis

Accounting for water type-specific bioavailability corrections, presents challenges and opportunities for metals. To this scope, recently developed aquatic Biotic Ligand Models can be used. These aquatic BLMs should be implemented in such a way that they can be used within a Decision Support System that assists in the interpretation of the (higher tiered) environmental quality criteria as set in the European Water Framework Directive. There are some major obstructions for a widespread, regulatory use of BLMs. Firstly, there is the conceptual complexity of the approach, requiring advanced chemical speciation calculations and normalization of toxicity data. Secondly, BLMs may require up to 10 measured input parameters, some of which are not readily available from standard monitoring programs. Thirdly, the power of BLMs to predict effects of metals on a field scale needs justification.

The aims of this PhD-thesis were:

1. to verify and optimize the ability of biotic ligand models to predict effects under realistic field conditions
2. to facilitate implementation of site-specific risk assessment methodology for several metals, based on mechanistic descriptions of biotic ligand models

The aims were subdivided in several research questions:

1. Is there a significant relationship between the calculated biotic ligand binding of metals and the measured bioaccumulation in aquatic species in the field?

2. How accurately do single metal BLMs, extended with a mixture model, predict toxicity of metals in a field setting?

3. What are vulnerable conditions and time periods for metal exposed ecosystems, based on changes in water chemistry and calculated effects on metal bioavailability?

4. Is it possible to derive a simplified function, based on a limited number of monitoring parameters, to facilitate widespread, practical use and implementation of BLMs without losing predictive capacity?

Set-up of this thesis

The reseach questions are answered in subsequent chapters.

In Chapter 2, it is tested if the BLM-concept is confirmed by bioaccumulation of metals in *Gammarus roeseli* and *Daphnia magna* exposed to metal mixtures in the field. Binding of metals to the organisms (as computed by the BLM) is expected to be closely related to accumulated body concentrations. However, due to chemical similarity some metals may share uptake pathways, for instance Zn and Cd, and interactions between metals are to be expected, which is not accounted for by the BLMs. In order to obtain additional evidence for validity of the BLM concept it is tested if the calculated occupancy of the biotic ligand is a better predictor for bioaccumulation than dissolved concentrations.

In Chapter 3, field and laboratory experiments were described to distinguish the effect of mixture toxicity from other factors such as temperature, food and oxygen on reproduction, bioaccumulation and growth of *Daphnia magna*. Exposure to multiple metals and other stressors is a challenge for risk assessment. Since BLMs are derived in a controlled single-metal exposure laboratory experiments, their performance under realistic conditions is unknown. The predictive power of the single metal BLMs extended with a mixture model was investigated by cage experiments in the field and parallel laboratory experiments using *Daphnia magna* as a model organism.

In Chapter 4, guidance is given on monitoring frequency for the ecological risk assessment. Field conditions are characterized by spatial and temporal variations in water chemistry, the presence of multiple contaminants and the presence of other, unknown stressors. Until now, geographical and temporal variation of bioavailability and species sensitivity only has been studied on a small scale on isolated samples, on single species or based on annual average concentrations of single metals. In this chapter the spatial and temporal variation of ecological risks

of Cu, Ni and Zn is quantified, to identify vulnerable conditions and time periods, based on changes in water chemistry and calculated effects on metal bioavailability.

In Chapter 5, BLM models are simplified by derivation of reliable and empirical linear equations for the calculation of site-specific hazard concentrations to 5% of species in an aquatic ecosystem (HC5). The metals most suitable for direct implementation in a risk assessment tool are Cu, Ni and Zn, because their BLMs are well described and extensive monitoring data are present. Moreover, toxicity data of many species and taxonomic groups are known which enables to observe the effect of changes in species sensitivities within the ecosystem. The simplified equations were chosen to initiate the development of a decision support system.