REVIEW

# Metal bioavailability and toxicity in freshwaters

**Danielly de Paiva Magalhães · Mônica Regina da Costa
Marques · Darcilio Fernandes Baptista ·
Daniel Forsin Buss**

**Abstract** Many factors influence metal speciation in freshwaters. Metal species that are more soluble are considered more bioavailable and toxic. However, evaluation of metal speciation in waters is a complex task. Moreover, the quantification of total and dissolved metals is not sufficient to determine toxic effects on the biota. Here, we review environmental parameters that influence metal bioavailability: mathematical models to predict toxicity, and the biological tools used to evaluate contamination in freshwaters ecosystems. The major points are the following: (1) we discuss many "exceptions" of chemical parameters that are deemed to increase metal bioavailability or to protect against metal uptake, such as pH and water hardness. We provide evidence of organisms and environmental conditions that break these rules and therefore should be considered when predicting impairment by metals. (2) We discuss the advances in mathematical modelling as a proxy to metal toxicity. (3) We discuss advantages and limitations of using multiple biological tools to assess toxicity, such as the use of biomarkers and microorganisms, zooplankton, benthic macroinvertebrates and fish communities. Biomarkers are efficient in detecting low concentrations of metals in a short-term exposition. Changes on biological community structure and composition are good tools to detect high metal concentration or chronic concentration in a long-term exposition. The use of multiple tools including chemical analyses and a set of biological indicators is recommended for a more accurate evaluation of metal impacts on freshwater systems.

**Keywords** Metal speciation · Biological responses · Models to predicting metal toxicity · Bioindicators · Biomonitoring · Biomarkers

D. de Paiva Magalhães (✉)
Programa de Pós-graduação em Química, (UERJ)/Laboratório de Ecotoxicologia Aplicada à Indústria Mínero-Metalúrgica, CETEM/MCTI, Av. Pedro Calmon, 900, Ilha da Cidade Universitária, Rio de Janeiro, RJ CEP 21941-908, Brazil
e-mail: dmagalhaes@cetem.gov.br; daniellypaiva@uol.com.br

M. R. da Costa Marques
Laboratório de Tecnologia Ambiental, Instituto de Química - Programa de Pós-graduação em Química, UERJ, R. São Francisco Xavier 524 - Maracanã, Rio de Janeiro, RJ CEP 20550-013, Brazil

D. F. Baptista · D. F. Buss
Laboratório de Avaliação e Promoção da Saúde Ambiental, IOC, FIOCRUZ, Av. Brasil 4365 – Manguinhos, Rio de Janeiro, RJ CEP 21045-900, Brazil

## Introduction

The basic principle of toxicology states that any substance can be toxic and the level of toxicity is dependent on the administered dose (Paracelsus, 1493–1541). The toxicity of metals does not depend only on the exposure dose, but also on chemical and physical speciation. Knowledge of the speciation of metals in water is essential in determining their potential toxicity and mobility in the environment (Forsberg et al. 2006; Ospina-Alvarez et al. 2014).

The term speciation refers to the occurrence of an element in different forms in a system (Templeton et al. 2000). The factors that influence the speciation of metals in freshwater ecosystems include: (1) ionic strength of the medium, (2) hardness of the water, (3) presence of organic matter, (4) pH, (5) redox potential and (6) its valence state. The combined action of all these factors could favour the formation of different metal species, resulting in a toxic metal with a higher or lower bioavailability (Bjerregaard and Anderson 2007).

Analysing metal concentrations of the water body or sediment is not sufficient to determine its toxicity. Adding biological effects to chemical knowledge provides a more effective diagnosis of environmental conditions (Munné et al. 2012). Several biological factors influence the bioavailability of metals, e.g. the route of exposure, the mechanism of sequestration and transport of metals by organic ligands and the exposed organism (Vijver et al. 2004; Wang and Rainbow 2008). In metal ecotoxicology, the term bioavailability has two meanings, one environmental and other toxicological. In the environment, bioavailability corresponds to the metal that is available for capture by a living organism and is integrated into its metabolic processes. Toxicological bioavailability is defined as the fraction of the concentration of the metal that is absorbed and/or adsorbed by the body. The assimilated fraction interacts with receptors and physiological sites fundamental to the body's metabolism, triggering toxic effects (Rainbow and Luoma 2011).

For aquatic animals, metal exposure can occur through two basic routes: by direct absorption through water and/or by feeding. For predators, such as some species of fish, amphibians and invertebrates, and animals that feed on debris, feeding is the primary route of exposure and accumulation of metals (Wang 2013). Although numerous studies have attempted to establish toxicokinetics of metals, the concentration accumulated in tissues of exposed animals is frequently not correlated to toxic effects. Inside the body, metals may chelate with various ligands, permanently or temporarily, leaving them not bioavailable to interfere in the metabolism (Wang 2013). Thus, it is difficult to estimates the toxic effect based on environmental or bioaccumulated concentrations. Due to the complexity of establishing toxicokinetic patterns, the use of biomonitoring tools, such as biomarkers and bioindicators, has proven to be a simpler and consistent methodology in determining environmental condition. It can be used as a tracking mechanism of toxicity and as an assessment tool for the toxicological impact of metals (da Cruz et al. 2007). Biomarkers are defined as a xenobiotically induced variation in cellular or biochemical components or processes, structures or functions that are measurable in a biological system or sample (Henderson et al. 1987). Bioindicators are defined as species or group of species that readily reflect the abiotic or biotic state of an environment, representing the impact of environmental change on a habitat, community or ecosystem or is indicative of the diversity of a subset of taxa or the whole diversity within an area (Gerhardt 2000). Biomonitoring the use of biological responses to asses and monitor the environmental health can be accomplished in situ through the investigation of aquatic biota, or in the laboratory, by applying microcosm techniques or acute and chronic toxicity bioassays (da Cruz et al. 2007).

This review aims to elucidate the key environmental and biological factors that contribute to metal toxicity, addressing the complexity of the formation of metal species in the aquatic environment and the importance to use both chemical and biological data in this assessment. This paper is divided into three parts. First, we discuss chemical interactions between metals and metalloids $Ag^+$, $Al^{3+}$, $Cd^{2+}$, $Cr^{3+}$, $Cr^{6+}$, $Cu^{2+}$, $Hg^{2+}$, methyl-Hg, $Pb^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ and various environmental parameters that influence its chemical speciation, solubility and bioavailability. Second, we analyse the usefulness and limitations of mathematical models that have been used to predict toxicity. Third, we examine biological responses triggered in exposed organisms and the use of these responses as biological tools to assess toxicity effects.

## Solubility, speciation and bioavailability of metals

In the freshwater environment, metals produce aggregates of different sizes. Nystrand et al. (2012) divide metals into three fractions: dissolved (<1 kDa), colloidal (between 1 kDa and 0.45 μm) and particulate (higher than 0.45 μm). The dissolved form is the most mobile and bioavailable and is generally the most toxic fraction. However, part of these dissolved metal ions may react with the sediment by adsorption, ion exchange with clay minerals and/or formation of complexes with oxyanions (Fig. 1a), which become less bioavailable (Bjerregaard and Andersen 2007). Colloids may behave in some respects as soluble matter—being more bioavailable and consequently being toxic to living organism—and in others as less soluble, therefore, not being directly available for microorganisms (Koukal et al. 2007). Colloids are effective in binding to trace metal, reducing the concentration of free molecules and its corresponding toxicity, but at the same time they can increase the bioavailability for specific organisms, e.g. filter-feeding organisms (Weltens et al. 2000). Metals associated with larger particles (<0.7 μm) are progressively concentrated in sediments, which act as temporary reservoirs (Vignati et al. 2006). Larger particles are generally not available to the biota, but modifications in water chemistry, e.g. increased salinity, reducing conditions, low pH and the presence of complex organic compounds (Fig. 1b), can cause its resolubilization (Butler et al. 2008; Bjerregaard and Andersen 2007; Gunkel-Grillon et al. 2014).

Influence of pH and redox potential on metal toxicity

The pH is probably the single most important variable that influences the behaviour of metals in the environment (USEPA 2007). The pH determines the degree of

**Fig. 1** Metal (M*) distribution between water and sediment and interference factors in solubility. 1 and 2 are exceptions for some metals as described in the text. *Boxes* with *letters* represent the steps mentioned in the text. $M_{alk}$—Alkali metals. $M_{alkEar}$—Alkaline earth metals
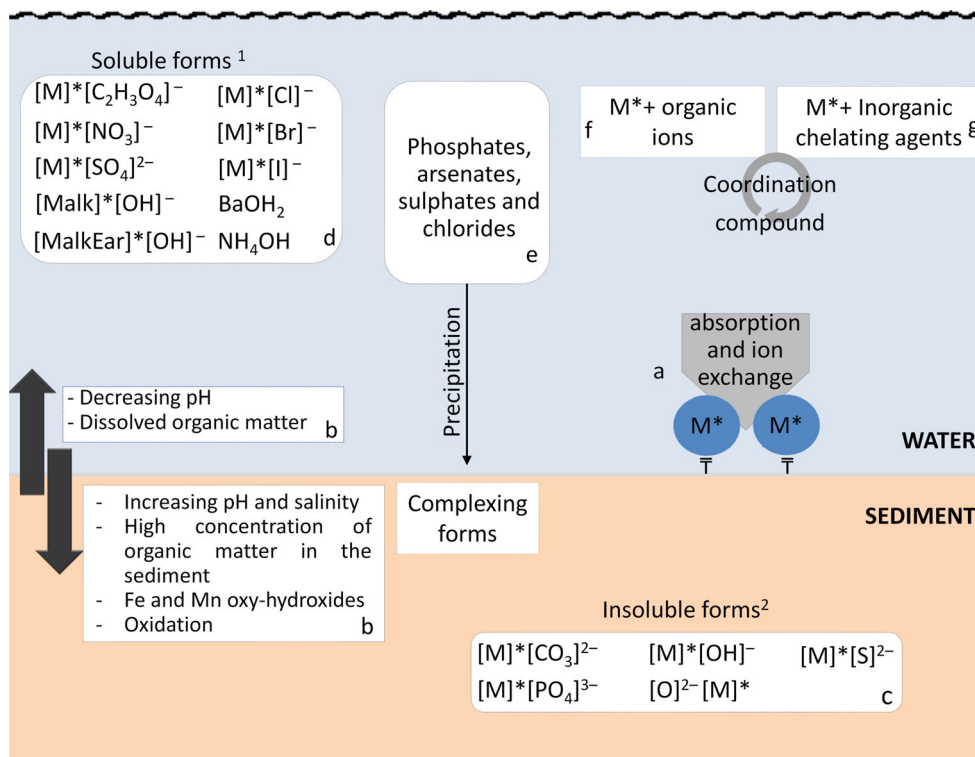


hydrolysis, polymerization, aggregation and precipitation, and proton competition for available ligands (Smith 2009). Generally, low pH induces the dissociation of metals, thus increasing their solubility, and consequently their toxicity, whereas in alkaline pH, metals precipitate as oxides and hydroxides, except for those consisting of alkali metal hydroxides, $Al^{3+}$ and $Ba^{2+}$ (Fig. 1b, c), becoming less bioavailable and less toxic (Cornelis and Nordberg 2007). Wilde et al. (2006) studying algae observed that lower pH increased the toxicity of $Cu^{2+}$ and $Zn^{2+}$. However, even being more soluble and bioavailable in acid pH, metals in some occasions can be less toxic in low pH, e.g. toxicity of Cd, Ni and Zn was lower at pH 6.3 for *Ceriodaphnia dubia*, *Pimephales promelas* and *Hyalella azteca* (Schubauer-Berigan et al. 1993); more toxic in alkaline conditions, e.g. toxicity of Cd, Ni and Zn was greater at pH 8.3 for *C. dubia, P. promelas* and *H. azteca (*Schubauer-Berigan et al. 1993) and $Pb^{2+}$ for fish (Grosell et al. 2006); or the pH can have no influence on metal toxicity, e.g. $Cd^{2+}$ for fish (Niyogi et al. 2008). Due to the competition between $H^+$ and metal at the gill surface, in low pH, $H^+$ may protect against metal uptake, while in higher pH there is a reduced competition with $H^+$, and thus, ionic metal is more available for uptake by the gill, despite a lower fraction of metal being available in the environment (Grosell et al. 2006; Niyogi et al. 2008).

Some metal species are strongly influenced by the redox potential. Under oxidizing conditions and above pH 5.5,

Cr, Cu, Hg, Mn and Fe react with water producing low solubility oxides and hydroxides. In reducing conditions, e.g. low concentration of dissolved oxygen, and pH <7, the solubility of metals increases by promoting soluble cations, thus favouring low oxidation number cations, for example, $Fe^{2+}$ instead of $Fe^{3+}$ (Weiner 2000). Metals $Al^{3+}$, $Ba^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ are not sensitive to oxidation and do not change their oxidation state under reducing conditions. The formation of oxides and hydroxides of these metals is correlated with pH (Weiner 2000).

Influence of water hardness on metal toxicity

Higher water hardness is generally associated with lower metal toxicity (Saglam et al. 2013). Ions $Ca^{2+}$ and $Mg^{2+}$ in the form of carbonates compete with other divalent metal ions for organisms' binding sites (Kozlova et al. 2009), functioning as blockers to the entry of metals in cells. Calcium ion also decreases gill permeability to ions, by binding to gill surfaces giving them a positive charge that repels other cations (McWilliams and Potts 1978). Water hardness associated with factors such as alkalinity and dissolved organic matter can change the speciation of metals and thus their toxicity (Heijerick et al. 2003) and bioaccumulation (Franklin et al. 2005). Some studies have shown that high water hardness has a more significant effect on acute toxicity than on chronic toxicity. That was observed for Zn toxicity for fish and invertebrates (De

Schamphelaere et al. 2005) and for Cu toxicity for *Daphnia* (De Schamphelaere and Janssen 2004). Also, there are metals that are more influenced by $Ca^{2+}$ than $Mg^{2+}$ concentration because they compete with $Ca^{2+}$ ligands, such as, $Cd^{2+}$, $Zn^{2+}$, $Pb^{2+}$ and $Co^{2+}$, so higher concentration of $Ca^{2+}$ function as a protection factor, decreasing the toxicity (Niyogi and Wood 2004; De Schamphelaere and Janssen 2004).

### Influence of alkalinity and salinity on metal toxicity

Water alkalinity is primarily determined by carbonate, bicarbonate and hydroxide ions. Phosphates, borates and silicates also contribute to alkalinity levels, although to a lesser extent. Higher water alkalinity decreases the toxicity of metal ions either by active surface competition for binding sites in tissues (Santore et al. 2001) or by reducing their concentration through the formation of precipitates that are insoluble (metal carbonates and phosphates, except those consisting of alkali and alkaline earth metals, Fig. 1c) (Cornelis and Nordberg 2007). This reduction in toxicity is more efficient in environments with higher salinity levels because the concentration of carbonate species increases with higher ionic strength. Additionally, higher salt concentration usually acts interfering on metal ions through the formation of metal complexes with anions, such as $CdCl^+$ or $CdCl_3^-$, forms that are less available for microbiota, reducing metals toxicity (Cornelis and Nordberg 2007).

### Influence of inorganic ions on metal toxicity

Most metals have high affinity to oxygen and sulphur ions, and thus, chemical forms of metals most commonly found in nature are binary compounds of oxide or sulphide. For example, hexavalent chromium is usually associated with oxygen, producing oxyacids like chromate ions ($CrO_4^{2-}$) or dichromate ($Cr_2O_7^{2-}$) (Singh et al. 2013). These forms are more soluble and more toxic for the aquatic biota than trivalent chromium, which is usually found in the form of insoluble oxides, hydroxides and phosphates that are preferably associated with the organic matter of sediments (Cervantes et al. 2001).

Soluble forms of metals have a greater potential to cause toxicity because they are more bioavailable. Generally, nitrates, acetates and all halides are soluble, except for some formed by $Ag^+$, $Hg^{2+}$ and $Pb^{2+}$ (Fig. 1d). Many metal sulphates are soluble, with the exception of $Ba^{2+}$, $Sr^{2+}$ and $Pb^{2+}$. In addition, $Na^+$ and $K^+$ salts are also soluble, with the exception of sodium antimony, potassium hexachloroplatinate and potassium cobaltinitrite (Fig. 1d) (Cornelis and Nordberg 2007).

The presence of phosphates, chlorides, sulphates and arsenates in water can cause the precipitation of metals, reducing its availability to organisms (Fig. 1e). Chloride ($Cl^-$) produces inorganic complexes in the environment, even at low concentrations (0.01 mol·$L^{-1}$). The resulting chlorine complex may interfere with the toxicity of metals. For example, high concentrations of $Cl^-$ decrease toxicity of $Cu^{2+}$, cause no interference in toxicity of $Cd^{2+}$ and increase toxicity significantly of $Hg^{2+}$ and $Pb^{2+}$ to *Escherichia coli* (Sarin et al. 2000).

### Influence of organic ions on metal toxicity

Organic ions can be of anthropogenic origin (e.g. complex agents contained in detergents) or can occur naturally in the form of humic and fulvic acids (Reeve 2002). Organic ions act as metal ligands, reducing the concentration of free metal cations and their bioavailability (Sánchez-Marín et al. 2007). This occurs because the complexation is the most important process of reaction between metal and organic ions, but adsorption and cation exchange reactions can also occur (Bezerra et al. 2009), producing coordination compounds (Fig. 1f). Coordination compounds are often less toxic than the original free metal because they have higher molecular weights and are frequently unable to pass through the biological membrane (Richards et al. 1999). Complexation occurs with hydroxyl, carboxylate, cyano and amino groups (Fig. 1f), and chelating agents such as inorganic carbonates, phosphates, cyanides and chlorides (Fig. 1g). For example, ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid reduce the accumulation and the toxicity of metals to fish (Klinck et al. 2005), bivalve molluscs (Funes et al. 2006), bioluminescent cyanobacterial (Rodea-Palomares et al. 2009), crustaceans (Borgmann et al. 2004) and unicellular algae (Vignati et al. 2010). Also, the toxicity of metals is frequently reduced with the increase in humic acid concentration due to its complexation properties (Rocha et al. 2000). Aquatic humic substances possess linking groups in which metals can be distributed between soluble and solid phases (Shanker et al. 2005). The stability of aquatic humic substances with metal species is determined by a number of factors, including the number of ligand atoms, the nature and concentration of metal ions, the concentration/characteristic of aquatic humic substances, the pH, complexation time, photodegradation, among others (Rocha et al. 2003; Rosa et al. 2002). This stability determines the transport mechanism, complexation, bioavailability and activity of metals in the environment. These interactions can be estimated using the model of Windermere Humic Aqueous Model VII (Tipping 1998), designed to calculate the chemical speciation balance in water, sediment, and surface and underground soils where the chemical composition is mostly of organic matter, such as humic and fulvic acids. The model simulates the influence of iron and

aluminium hydroxide precipitation, of cationic exchanges with clay mineral, and the adsorption–desorption reactions of fulvic and humic acids (Tipping et al. 2011). The model, however, has some limitations. Baken et al. (2011) observed that metal complexation in waters with anthropogenic discharges was larger than that estimated with Windermere Humic Aqueous Model, because the model only takes into account binding on humic substances. Furthermore, not all metal-organic complexes are biologically unavailable. If the metal is chelated to lipophilic organic ligands, it can diffuse passively through the cell membrane and causing toxic effects (Fig. 2a). Toxicity tests with Cd in the presence of some of natural organic matter increased Cd toxicity to rainbow trout compared to Cd-only controls (Schwartz et al. 2004). For the photobacterium *Vibrio fischeri*, the toxicity of $Pb^{2+}$ was increased in the presence of humic acids, but it was dependent on the concentration. In concentrations of Pb below 1 mg/L, humic acids caused a relatively high increase in metal toxicity, but for Pb concentrations higher than 1 mg/L, the toxicity was almost constant (Tsiridis et al. 2006). The interpretation of the complexation mechanism of heavy metals in the presence of organic matter and the consequent changes in their toxicity and bioaccumulation are complex and it dependent on metal concentration and type of organic matter and with organisms exposed.

## Influence of iron, manganese, sulphur and redox potential on metal toxicity

The oxidation–reduction (redox) transformations in aquatic environment may lead to solubilization or deposition of metal ions in the sediment. Among the metal ligands that can immobilize them, the oxy-hydroxides of Fe and Mn and sulphides have been highly recognized (Fig. 1b) (Gasparatos 2013). Under oxidizing conditions, hydrated oxides of iron and manganese strongly absorb and precipitate other metals such as Cu, Pb, Ni, Co and Cr, thus removing these elements from water, but this state can be reversible if the environment becomes reduced (Matagi et al. 1998). In this case, soluble compounds of iron and manganese can produce insoluble sulphides (Fig. 1c) (Kosolapov et al. 2004) that act to immobilize metals in sediment, altering their speciation and decreasing their bioavailability. Thus, even in very impacted environments, high concentrations of sulphide could reduce metal toxicity by precipitation, e.g. for cadmium, copper, lead, nickel and zinc (Berry et al. 1996). Marcussen et al. (2008) evaluated the behaviour of 33 compounds in freshwater and found that sulphide precipitation was the main mechanism of retention of these compounds in sediments under reduced conditions.

Analyses of acid-volatile sulphide are used in the extrapolation of the bioavailability of metals in sediments



**Fig. 2** Mechanisms of assimilation and detoxification of metals (M*) in cells. *a* Lipophilic metal forms passively diffuse through the plasma membrane; *b* hydrophilic forms require active transport into the internal environment; *c* inside the cell, metal is oxidized or reduced by reactive species; *d* that can cause DNA damage or oxidative stress by activating enzyme glutathione peroxidase (GSH-Px); *e* it can be further stabilized by proteins, such as metallothionein (MTs); *f* chelated by proteins and stored in organelles; *g* linked to proteins, amino acids, and organic acids; *h* or complexed in the extracellular medium; *i* when in excess, metals are expelled out of the cell
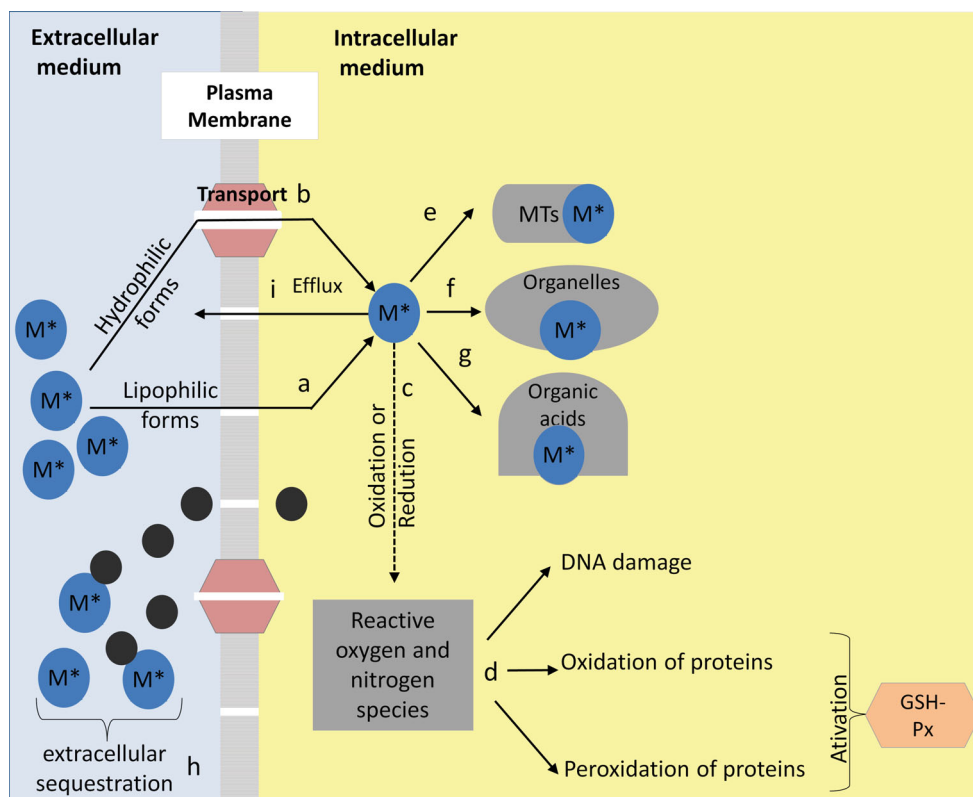
in biomonitoring programs (Oueslati et al. 2010). Han et al. (2005) observed that in sediments where the sum of simultaneously extracted metals is less than or equal to acid-volatile sulphide, no adverse effects or impacts were observed to the amphipod *Grandidierella japonica*, suggesting that acid-volatile sulphide normalization is useful to predicting non-toxicity. However, metals associated with acid-volatile sulphide may be released within sediments through storms, dredging activities, oxidation, bioturbation, etc., and may have adverse environmental impacts (Prica et al. 2008). When the sum of the ratio between simultaneously extracted metals (SEM) and acid-volatile sulphide (AVS) is <1 ($\Sigma$SEM/AVS < 1), the concentration of metals in interstitial water is negligible, and the bioavailability of metals minimized (Allen et al. 1993). Sediments with sum of the ratio between SEM and AVS are higher than 1 ($\Sigma$SEM/AVS > 1) not necessarily will increase metal toxicity because there are many other metal-binding phases in sediments. The AVS pattern might be influenced by seasonal variations (Prica et al. 2008), decreasing in summer due to the metabolization of sulphides by microorganisms—this is the more susceptible period to toxic effects of metals on biota.

## Models developed to predict metal toxicity

As described in this review, many factors influence the environmental bioavailability of metals. Even not knowing the fraction of metal bioavailable, data on dissolved metals together with other water chemical and physicochemical parameters can be used in computational models to predict the available fraction and toxicity to some aquatic organisms.

The Free Ion Activity Model proposed in the 1970s was one of the first models used to explain metal–organism interaction (Campbell 1995). Free Ion Activity Model is based on the concept that metal toxicity is caused mainly by free metal ion concentrations (rather than total metal concentration) that is recognized as the most important species permeating through biological membranes (Pesavento et al. 2009). Free metal ions produce hydrophilic compounds, i.e. free hydrated metal that could be transported across biological membranes by endocytosis, diffusion, facilitated diffusion (usually lighter metals belonging to group of d-block with affinity for S- or N-ligands in proteins) or by active transport that requires energy expenditure (usually heavy ions from groups of s- or p-block that do not bind to protein carriers and transport against the concentration gradient) (Fig. 2b). Free Ion Activity Model is a chemical equilibrium model that considers two main processes: the kinetic exchange on the cell surface of the free ion metal species and the internalization

(Wang 2013). This model is used only to predict the uptake/bioavailability of the metal instead of its toxicity, because it only deals with the transport of the metal across the biological membrane.

The Biotic Ligand Model partially corrects these limitations of the Free Ion Activity Model. Both models share the same concepts, but Biotic Ligand Model provides a better understanding because it simultaneously takes into account the geochemical speciation, as well as the relative binding (or not) of metal species to the site of toxicity (Niyogi and Wood 2004; CCME 2007). The development of this model is relatively complex, and Hering (2009) warns that Biotic Ligand Model data need to be scrutinized for confounding factors. The model requires the known toxic metal concentrations in the biotic ligands (e.g. fish gills) and the binding stability constant to calculate the likely toxic metal free ion concentrations in the environment and the likely toxic metal concentrations by considering metal complexation by dissolved organic matter and competition, such as with Ca, Mg and $H^+$ (Wang 2013). However, once developed the Biotic Ligand Model requires a minimal input of data, which can be acquired using non-sophisticated analytical instrumentation, such as the concentration of soluble metals that is under investigation, Ca, Mg, dissolved organic carbon and pH. At present, the model was developed only for some organisms and for a few metals, such as $Cu^{2+}$, $Ag^+$, $Zn^{2+}$ and $Ni^{2+}$ (Wang 2013). The Biotic Ligand Model was first designed to predict acute toxicity to fish (i.e. 48-h $LC_{50}$) and was empirically calibrated to predict toxicity to aquatic invertebrates (daphnids) and algae (Niyogi and Wood 2004).

The Biotic Ligand Model has been used to predict metal toxicity for laboratory, and more recently, its precision was investigated for field conditions. Allen and Jansen (2006) evaluated several studies about copper acute toxicity in the field and showed that the toxicity predicted by Biotic Ligand Model varied within two standard deviations of the mean observed acute toxicity (48-h $LC_{50}$). This amount of deviation was considered satisfactory, and some Biotic Ligand Model have been applied by environmental agencies to predict toxicity in the field. In the USA, the most advanced Biotic Ligand Model is for the acute toxicity of copper (Di Toro et al. 2001; Santore et al. 2001) and was incorporated by US Environmental Protection Agency in Water Quality Criteria (USEPA 2007). Countries in the European Union are currently using Biotic Ligand Model to risk assessments of zinc, nickel and copper (Allen and Jansen 2006), and Environment Canada also adopted the Biotic Ligand Model to understand patterns of metal bioaccumulation (CCME 2012).

The Biotic Ligand Model predicts metal toxicity by the ability of the metal to bind in cell surface, based in the premises that after binding, the metal will be incorporated

into the cell and cause a toxic effect. However, inside the cell, the metal could be associated with some fraction that not necessarily will cause toxic effects. In order to evaluate the toxicity from accumulated metals in the subcellular components of organisms, the Subcellular Partitioning Model was developed. The premise of this model is that the total concentration of metals in biological tissues cannot estimate metal toxicity due to the complexity of metals that link to biotic ligands (Wang and Rainbow 2008). The Subcellular Partitioning Model calculates the quantity of metal in five cell fractions: cellular debris, organelles, metal-rich granules, proteins denatured by heat and heat-stable proteins, using differential centrifugation and physical and chemical treatments (Blackmore and Wang 2002; Wallace and Lopez 1996; Wallace et al. 2003; Wallace and Luoma 2003). The proteins denatured by heat and organelles fractions are the most sensitive fractions to metal toxicity and are considered main targets of metal attack in cells, whereas metal-rich granules and heat-stable proteins are biological detoxification fractions and function by reducing toxicity (Wallace et al. 2003). These methods are routinely used in determining the distribution of metals in subcellular fractions and in identifying which metal fraction is more related to toxicity and leaves metal more bioavailable and toxic. This means that the metal concentration present within the cell is not necessarily the concentration that causes a toxic effect. Environment Canada applies Subcellular Partitioning Model together with Biotic Ligand Model to determine metal bioaccumulation (CCME 2012). One limitation of this model is that it can only be applied when exposition is based on sublethal or chronic concentrations, since it does not respond well to acute concentrations. Tsui and Wang (2006) found that a correlation between the most sensitive fraction and $Hg^{2+}$ exposure was only observed in sublethal concentrations causing <1 % mortality, while for acute exposition the use of total body concentration had a better correlation with toxic effects than its subcellular compounds.

Free Ion Activity Model, Biotic Ligand Model and Subcellular Partitioning Model are based on the effect of a single metal in a specific organism, and it has been a challenge to develop models for mixture of metals. Two models have been developed for mixtures of multiple substances (not only metals): the Concentration Addition Model, used for mixture of substances with similar mechanism of action that neither interact on a physicochemical level nor in their toxicokinetics and toxicodynamics, and the Independent Action Model, that assumes that the mixture components act on different subsystems—tissues, cells, molecular receptors—of an exposed organism and that impaired subsystems affect the end point under observation, independently of each other (Backhaus and Faust 2012). Comparing Concentration Addition Model

and Independent Action Model, Lock and Jansen (2002) found that observed effects were lower than the effect predicted by the Concentration Addition Model and higher than the predicted by Independent Action Model for all metal mixtures tested, which means that Independent Action Model is not indicated to predict risk because it underestimates toxicity, while Concentration Addition Model tends to overestimate the risk. Similar results were observed by Norwood et al. (2003) that evaluated mixtures of metals using the Concentration Addition Model and found that for 70 % of the cases, effects predicted by Concentration Addition Model were equal to or higher than the observed effects (43 % less than additive; 27 % strictly additive), while for 30 % of mixtures the effect was underestimated by the model. Although some metals have different ligands and mechanism of action—which means the Independent Action Model would be more indicated to assess toxicity—according to Sharma et al. (1999), toxicity of complex mixtures (i.e. those containing many toxicants with different modes of action at subtoxic concentrations) is often more or less conveniently predicted by toxic unit summation. Thus, Concentration Addition Model is considered a conservative model that represents a reasonable worst-case scenario for the risk assessment of metal mixtures in ecosystems. Concentration Addition Model and Independent Action Model are based on acute effects of each individual metal present in the mixture, and their limitation is not considering interactions between the components in a mixture.

Other models have been specifically developed to predict the toxicity of metal mixtures. Many of those were inspired in the *modus operandi* of Concentration Addition Model, by summing the individual effect of each metal, using the Biotic Ligand Model or the Windermere Humic Aqueous Model (WHAM) (e.g. Chen et al. 2009; Iwasaki et al. 2014; Jho et al. 2011; Kamo and Nagai 2008; Playle 2004). Thus, these models assume the limitations of previous models, being unable to address all aspects involved in the determination of metal toxicity. The toxicity of a metal mixture is a result of the reaction among metals and other components in the mixture, the uptake fraction, its distribution in the organism and metabolization. Most of those models were performed in laboratory with mixtures of few metals and were based on the assumption that the different toxic metals share the same biotic ligand that is available for calcium uptake. They assume that the organisms will die due hypocalcemia when so few ligands are available for calcium uptake. The toxic mechanisms of heavy metals are not fully understood, but hypocalcemia is suggested to be the most likely toxic mechanism for some toxic metals (Kamo and Nagai 2008). However, not all metals will compete principally for $Ca^{2+}$ ligands. Playle (2004) simulated a model using two-to-six metal mixtures

(Ag, $Cu^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Pb^{2+}$ and $Zn^{2+}$) by combining Biotic Ligand Model and the concept of toxic unit, using the idea of Concentration Addition Model. Some simulations yielded greater than strict additive at low metal concentrations, strict additive at intermediate metal concentrations and less than strict additive at high metal levels, independently of metal combinations. For mixtures with high metal concentrations, the author found a strong competition for binding sites, and thus, the observed effect was lower than the expected (Playle 2004), which, in this case, could be explained by the use of metals that were $Ca^{2+}$ antagonists, such as $Cd^{2+}$, $Zn^{2+}$, $Pb^{2+}$ and $Co^{2+}$, and $Na^+$ antagonists, such as $Cu^{2+}$ and $Ag^+$, and considered that all metals would compete for the $Ca^{2+}$ ligands. Metals that interact at common sites should follow principles of Concentration Addition Model, and those acting at different sites should exhibit additive effects (Niyogi and Wood 2004).

The applicability of Biotic Ligand Model to assess environmental mixtures is limited, first because there is no Biotic Ligand Model for some metals and second because, in general, environmental concentration is low, characterizing a chronic exposition, which violates the equilibrium assumptions of current Biotic Ligand Model for some metals, such as $Cu^{2+}$ and $Ag^+$. In the case of $Ag^+$, the chronic toxicity mechanism is similar to the acute mechanism, i.e. affecting $Na^+$ balance (Brauner and Wood 2002). However, in this case, the protective action against acute toxicity produced by some factors, such as $Ca^{2+}$, $Mg^{2+}$, dissolved organic matter, pH and $Cl^-$, are either lessened or non-existent during chronic exposure for $Cu^{2+}$ and $Ag^+$ (De Schamphelaere et al. 2004; Brauner and Wood 2002a, b; Brauner et al. 2003). Thus, there is an urgency to develop Biotic Ligand Model that predicts chronic toxicity. Niyogi and Wood (2004) provided an overview of chronic Biotic Ligand Model for individual metals. Schmidt et al. (2010) developed the model chronic criterion accumulation ratio, to assess the toxic effect of chronic metal concentrations on macroinvertebrate populations in streams. This study was based on the idea of Biotic Ligand Model and the "Water Quality Criterion Continuous Concentration" developed by the USEPA (2009). Although the study of Schmidt et al. (2010) has shown good correlation between chronic criterion accumulation ratio and total taxa richness of macroinvertebrate populations in the field, their model did not consider the effect of some metals that were found in the studied stream, such as Al, Fe, Mn or Pb, because there was no Biotic Ligand Model available for these metals. Including this information could help increase the sensitivity of the model to predict effects of metals in the environment.

More recently, the model "Quantitative Ion Character-Activity Relationships-Species Sensitivity Distributions"

(QICAR-SSD; Mu et al. 2014) was developed to predict chronic effects of metal mixtures. The model derived the water quality criterion continuous concentration of 34 metal and metalloids to eight aquatic organisms, combining water chemical data with values of chronic toxicity tests (NOEC) found in the literature. The prediction error was within 100-fold the observed effects, and the authors concluded that the model is a promising tool, but needs improvement with some aspects related with metal toxicity (e.g. environmental behaviour, exposure component and external environmental characteristics).

The Windermere Humic Aqueous Model-Toxicity Function (WHAM-FTOX) model was also used in field assessments. It is based on WHAM both to calculate aqueous chemical speciation and to estimate the accumulation of protons and metals by freshwater organisms. The toxicity of cations is quantified by a linear toxicity function (FTOX) (Stockdale et al. 2010). The model is similar to Biotic Ligand Model, but it also considers some environmental interferents, like competition effect among cations. In WHAM-FTOX, the accumulation of reactants, such as $H^+$ and metal cations, by the organism is non-specific, whereas in the Biotic Ligand Model toxicity depends upon the extent of occupation of a key site, such as the biotic ligand (Stockdale et al. 2010). WHAM-FTOX model was applied in field to predict changes in macroinvertebrate species richness, specifically of Ephemeroptera, Plecoptera and Trichoptera (Stockdale et al. 2010) and zooplankton richness (Stockdale et al. 2014). In these studies, the authors compared the richness species predicted by the model with measured field richness and metal accumulation in organisms and found good correlation when values were log transformed. In general, observed species richness is lower than predicted, but in some instances agreement is close and is rarely higher than predictions (Stockdale et al. 2014). Thus, it seems that this model generally underestimates the effects in the field, or maybe, there are other interferents that could be acting to promote the reduction of species richness. In mixtures, some metals could inhibit or favour the assimilation of other metals (Franklin et al. 2002). The exact proportion of inhibition or assimilation is not possible to be determined. Metal assimilation will depend on metal concentration, interactions between metals, the exposed organism and water chemistry (Komjarova and Blust 2009a, b, c; Vijver et al. 2004). In the field, it is not possible to quantify all components of a mixture, and how the presence of unknown substances can interfere with metals and in the predicted toxic response. Because of those limitations, many environmental agencies assess metal contamination by their individual effects, using the quantification of individual metal and limiting their discharge according to their priority substances lists, and for some metals, Biotic Ligand Model is used, except for

Australia and New Zealand that use toxic units summation with a threshold of 5 (ANZECC 2000).

The future of metal ecotoxicology moves towards building robust models that express the influence of chemical speciation, metal interactions and metal toxicokinetics and toxicodynamics. Such models will allow to estimate the potential effect of metal mixtures, but they will not replace ecotoxicological tests, in situ biomonitoring or metabolic assessment, because of the complexity of biological systems.

## Biological tools for assessing metal toxicity

Chemical analyses of the water or sediment are the most direct approach to reveal metal pollution status in the environment, but it does not provide evidence of toxicity for organisms or the ecosystem (Zhou et al. 2008). The use of biological responses to asses and monitor environmental health, referred as "biomonitoring", has the advantage to integrate organisms' responses to multiple effects of metals and its interactions with environmental factors in all routes of exposure. It can be performed by exposing organisms to environmental samples in laboratory (ex situ) or by observing responses of the biota in the environment (in situ).

Different levels of biological organization can be used to evaluate biological effects of metals. The first observed toxic response occurs in biomarkers—xenobiotically induced variation in cellular or biochemical components or processes, structures or functions. Biomarkers can provide identifiable response to low metal concentration and in short exposure time, for example, the inactivation or activation of proteins (Siripornadulsil et al. 2002) and the induction of oxidative stress with subsequent cell damage (Sinha et al. 2003). The binding of certain metals onto binding sites on the cell wall can lead to cell surface disruption, affecting cellular morphology and metabolism, which in turn may lead to death (Sinha et al. 2003; Vignati et al. 2010). The death of sensitive organisms and changes in community structure and composition are a secondary response and are efficient bioindicators at high metal concentration or in case of chronic expositions (long time of exposition to low concentrations of metal).

### Biomarkers and other strategies of organic defences

Many physiological parameters have been used to establish a relationship between exposure to metals and their effect in the health of exposed organisms. Among them, the evaluation of oxidative stress, metallothionein activity and enzyme delta‐aminolevulinic acid dehydratase has been used as biomarkers for metals. Metal accumulation in

organisms can cause oxidative stress generated by reactive oxygen species (Fig. 2c), resulting deleterious damages in cells (Pinto et al. 2003; Livingstone 2001). As a response to oxidative stress caused by metals, animal cells increase glutathione metabolism (glutathione peroxidise, glutathione S-transferase, glutathione reductase, reduced glutathione, oxidized glutathione) and other oxidative antioxidant enzymes (Fig. 2d). Changes in the ratio between total glutathione and oxidized glutathione have been used in vivo as biomarkers of metal toxicity (Carney Almroth et al. 2008).

The response of the antioxidant system varies depending on the metal or mixture of metals, the tissue and the time and type of exposure (Atli and Canli 2010). Also, the concentration of these enzymes in tissues may vary for each fish species, and thus, some tissues are more indicated to evaluate the effect of certain metals. Monteiro et al. (2010), working with HgCl sublethal concentrations, showed that liver, gills and heart of fish matrinxã (Brycon amazonicus) had higher levels of glutathione peroxidise, glutathione S-transferase and glutathione reductase activity, while reduced glutathione and oxidized glutathione levels increased in heart muscle; in white muscle, they found a decrease in reduced glutathione levels and an increase in oxidized glutathione content. Nunes et al. (2014) found that lead, copper and cadmium chronic exposure caused a significant dose-dependent increase in glutathione S-transferase activity in gill tissue of European eel, Anguilla anguilla and that zinc increased glutathione S-transferase in liver tissue. Also, the time of exposure was shown to interfere in enzymes activity level. Eroglu et al. (2014) found a higher activity of reduced glutathione and glutathione S-transferase in the liver of freshwater fish Oreochromis niloticus after 7 days of exposition to metals Cd, Cu, Cr, Pb and Zn, but after 14 days of exposition enzyme activity returned to normal values. In addition to enzymes, genes expression of the glutathione S-transferase family can also be used as biomarkers. Zhang et al. (2012) found a significant correlation between seven genes of the glutathione S-transferase family from Venerupis philippinarum with cadmium and copper exposition.

Glutathione metabolism can also be triggered by other environmental stressors, such as the presence of persistent organic pollutants, pesticides, toxins, pharmaceuticals and nanomaterials, as well as changes in water temperature or oxygen concentration. These responses are also dependent on the species, age, sex and reproductive cycle (Hellou et al. 2012). For this reason, combining these responses with other biomarkers and chemical analysis helps building evidence on the effect for ecosystem health.

Another strategy of organisms to avoid metal toxicity is to thermostabilize it, chelating the metal to proteins such as metallothionein (MT)—and its variants—present in

eukaryotic invertebrates and vertebrates (Fig. 2e) (Vijver et al. 2004). The quantification of these proteins and the analysis of mRNA expression of metallothionein genes can be used as a biomarker of metal exposure (Sevcikova et al. 2011). This group of enzymes is the most used biomarker of metal exposure because it is related to the regulation of essential metals and detoxification of excess amounts of metals intracellularly (essential metals and also of non-essential toxic metals). There is a wide range of metals capable of binding to metallothionein. Most metallic ions belonging to group 11 and group 12 of the periodic table are known to bind to cysteine SH groups (Amiard et al. 2006). In plants, phytochelatins are important metal-binding proteins (Nagajyoti et al. 2010).

Other factors may influence the level of metallothionein, such as the surrounding medium, the physiology of each species and also differences among individuals of the same species (Amiard et al. 2006). Thus, they must be considered when using this biomarker to detect metal pollution. Metallothionein production could be induced in organisms exposed to other contaminants, such as antibiotics, vitamins, polycyclic aromatic hydrocarbons or herbicides (Machado et al. 2014; Mosleh et al. 2004; Raftopoulou et al. 2006; Templeton and Cherian 1991) and to environmental factors such as starvation, anoxia, freezing and salinity (Baer and Thomas 1990; English and Storey 2003), but the level of induction is usually lower than those caused by metals (Kägi 1993). With so many confounding factors, the use of more than one type of biomarker is recommended to confirm the relationship between environmental stressors and ecological effects caused by metals (Hagger et al. 2006). In many cases, the use of antioxidant system parameters and metallothionein levels is recommended. There is no clear relationship between these biomarkers (Eroglu et al. 2014), so alterations on both can confirm metal exposition. For example, Velma and Tchounwou (2010) found an increase in the antioxidant enzyme activity in kidney and liver of fish *C. auratus* exposed to $Cr^{+6}$—indicating oxidative stress—and also found an increase in metallothionein levels. A biomarker that is less affected by confounding factors is the quantification of metallothionein mRNA, which shows no correlation with fish age, sex or sampling location (Laurie 2004). Another advantage of this method is that its response can be observed in short-term exposure, e.g. in 48 h (Laurie 2004). Quantitative analysis of mRNA expression of metallothionein genes could also be appropriate in case of high levels of metals and when there is no evidence of oxidative damage in fish tissues (Sevcikova et al. 2011).

The enzyme delta-aminolevulinic acid dehydratase is a biomarker of lead exposure, indicating effects even at very low concentrations. Delta-aminolevulinic acid dehydratase can be found in vertebrates, invertebrates, bacteria and plants, and it is a precursor of chlorophyll molecules (Gonçalves et al. 2009; Zaccaro et al. 2001). Lead inhibits enzymes delta-aminolevulinic acid dehydratase, coproporphyrinogen decarboxylase and ferrochelatase. Thus, the substrate of these reactions (i.e. delta-aminolevulinic acid, coproporphyrinogen III, protoporphyrin) accumulates in blood and urine (Alcedo and Wetterhahn 1990; Ho 1990). Some authors proposed the calculation of a "reactivation index" for delta-aminolevulinic acid dehydratase. This method is sensitive for assessing delta-aminolevulinic acid dehydratase inhibition, especially for samples with high variability, or when low inhibition values are observed (Rodrigues et al. 1996). A strong correlation has been reported between the concentration of lead in blood of *Prochilodus lineatus* fish and delta-aminolevulinic acid dehydratase reactivated in environments contaminated by domestic sewage (Lombardi et al. 2010).

Another biological protective mechanism against metal toxicity is its accumulation in organelles in the form of inert granules (Fig. 2f), which are excreted or deposited during the life cycle (Vijver et al. 2004). $Cd^{2+}$ and $Hg^{2+}$ could be converted to covalent organometallic compounds that behave in a manner similar to covalent organic compounds and bioaccumulate in adipose tissues. Marasinghe Wadige et al. (2014) showed that for freshwater bivalve *Hyridella australis*, 83–91 % of lead accumulated in hepatopancreas was detoxified and stored in metal-rich granules. The proportion and concentration of metal in this fraction increased with exposure. These granules can be directly measured in tissues (Reeve 2002).

Effects on aquatic biota and bioindicators

Regarding metal toxicity effects, various groups of aquatic organisms have been studied to describe routes of exposure and toxic effects. These effects range from the determination of lethal concentration and no observed effect concentration using ecotoxicological tests, to physiological and structural changes in biological communities, which can only be observed after high metal concentration pollution or chronic exposure. The bioindicator to be used depends on the purpose of the research and on the extent of damage of the studied environment.

A change in the biota is an effective method to evaluate the impact of environmental pollution because it reflects integrated effects of mixtures of chemicals. Among the major groups of organisms used as bioindicators, the most commonly used are microorganisms (including bacteria, Protista including microalgae, diatoms and yeasts), zooplankton, benthic macroinvertebrates (insects, crustaceans, molluscs, annelids) and fish, representing different trophic levels and ecosystem functions.

## Microorganisms

The first site of contact between a metal and a microorganism is the cell wall, which consists of lipids, proteins and polysaccharides. These biopolymers contain different functional groups such as imidazole, thioether, carboxylic acid, hydroxyl, carbonyl, phosphate and phenol, which have the property of producing coordination complexes with metals, thus enabling its adsorption (Al-Rub et al. 2004, 2006). To protect against the toxic action of metals, microorganisms have developed several mechanisms including:

- Intracellular reactions with chelating agents that transform metal ions in less bioavailable forms (Fig. 2d–g). Complexing agents including glutathione, amino acids, phytochelatin (Clemens 2001; Schat et al. 2002), metallothionein, organic acids (El-Enany and Issa 2001; Oven et al. 2002) and thioredoxin (TRX) (Lemaire et al. 1999).
- Efflux or expulsion of metal to the external medium to reduce the excessive level of metals in the cytoplasm (Lee et al. 1996; Robinson et al. 1993) and to decrease the flow of inert metal complexes (Fig. 2i);
- Internal storage compartments such as vacuoles and chloroplasts after being complexed and mediated by transport proteins (Fig. 2f) (Escher and Hermens 2004; Nishikawa et al. 2003; Tamas and Wysocki 2001); metals like Hg, Ag, Pb and Ni are bioaccumulative, and they neither break down in the environment nor easily metabolized. Such metals accumulate in ecological food chain through uptake at primary producer level, such as microalgae, and then through consumption at consumer levels (Nagajyoti et al. 2010).
- Extracellular sequestration—microorganisms are capable of excreting compounds that will sequester metal ion in the extracellular medium (Fig. 2h) (Ahner et al. 1998; Macfie and Welbourn 2000), reducing its environmental bioavailability (Kurek et al. 1991). Smiejan et al. (2003) observed that bioaccumulation of $Cd^{2+}$ by *Rhodospirillum rubrum* was reduced by the production of extracellular ligands. McKnight and Morel (1979) observed that algae produce extracellular sequestering agents such as polysaccharides, proteins, peptides and small organic acids that are capable of decreasing bioavailable metal concentrations in the medium surrounding the cell.

The use of microorganisms in biomonitoring assessment includes laboratory toxicity tests and evaluation in situ. In laboratory, Magalhães et al. (2014) tested chronic toxicity bioassays with *Chlorella vulgaris*, *Pseudokirchneriella subcapitata* (and also invertebrates Cladocera and fish) for ten individual metals and metalloids ($Ag^+$, $Cd^{2+}$, $Cu^+$,

$Cu^{2+}$, $Cd^{2+}$, $Cr^{3+}$, $Cr^{6+}$, $Ni^{2+}$, $Zn^{2+}$ and Hg) and mixtures (simulated effluent and siderurgic effluents). *C. vulgaris* bioassays were more sensitive in most of cases than *P. subcapitata*, except for $Cr^{3+}$. Thus, chronic toxicity bioassay with *C. vulgaris* was a good tool to detect metal toxicity under laboratory conditions. In situ biomonitoring comprises the evaluation of changes on community composition, standing crop, biomass (usually measured for algae as chlorophyll a), changes on cell morphology and, when possible, the quantity of metal accumulated into the organism.

Community composition evaluation is based on the presence/absence of tolerant/sensitive taxa to metal pollution. Monteiro et al. (1995) observed the change in algae community composition of Sado River in Portugal, with species *Rhodomonas minuta*, *Synedra ulna*, *Crucigenia tetrapedia* and *Stephanodiscus hantzschii* being replaced by more resistant algae (*Gomphonema parvulum*, *Scenedesmus armatus* and *Nitzschia frustulum*) due to contamination of pyrite mines effluents (88 µg l$^{-1}$ Cu, 2.6 µg l$^{-1}$ Cd and 1,800 µg l$^{-1}$ Zn). The use of algae community composition is more indicated after long-term exposition, because in acute disturbance most algae recover quickly (USEPA 1995). Standing crop and biomass (usually measured as chlorophyll a) are also typically assessed, but CCME (2012) observed that algal biomass is not a reliable indicator of metal effects, since other factors could interfere, for example, long-term shifts in species composition, reduction in grazing pressure, excessive nutrient loads and sedimentation/turbidity (CCME 2012).

Numerous studies have investigated metal impacts on periphyton communities, the majority of studies being devoted to diatom communities. Those species tolerate high metal concentrations, and also when metal concentrations in water are too low to be detectable by routine analyses, they can provide information on metal contamination through bioaccumulation or abnormal cell morphology cause by metals (Duong et al. 2008; Lavoie et al. 2012; Pandey et al. 2014). Lavoie et al. (2012) observed highly significant relationships between free metal ion concentrations in water and intracellular metal and phytochelatin.

## Zooplankton

Zooplanktonic species (especially cladocerans and copepods) are widely used to assess water quality due to their ecological relevance in the food chain, high sensitivity, short generation time, and high fecundity and population growth rate. These species can accumulate dissolved metals directly from the water column or assimilate particulate-associated metals during dietary ingestion (Sofyan et al. 2006). Generally, metals dissolved in water are more

likely to be deposited in gills and external tissues, while metals acquired from food sources are deposited in internal tissues (Hook and Fisher 2001a, b; Munger and Hare 1997), but the mechanisms of toxicity remain largely unknown (Hook and Fisher 2001a, b).

In microcrustaceans, a fraction of the whole-body burden of metals (19–97 %, depending on metal species and organisms, Keteles and Fleeger 2001) is associated with their chitinous exoskeleton. Metals may either adsorb to the surface of the exoskeleton or bind to the inner exoskeleton matrix after uptake and transport through the hemolymph. Metals in exoskeleton are slowly absorbed and diffused into the body (Keteles and Fleeger 2001). Because the adsorption by the exoskeleton is faster than the diffusion of metal inside the body, these animals can protect themselves from contamination by changing their exoskeleton, which occurs on average every 2 days, depending on the species. With high adsorption of metals in the exoskeleton, and being universal preys, zooplankton represents a source of trophic magnification in ecosystems. When ingested, metals adsorbed in the exoskeleton dissolve due to low pH and complexing conditions of the digestive tract of predators (Robinson et al. 2003), becoming more bioavailable.

Among the various species of zooplankton, cladocerans *Daphnia magna*, *Daphnia pulex*, *Daphnia similis*, *Moina macrocopa*, *Moina micrura* and *C. dubia* have been widely used in ecotoxicological studies around the world (Stankovic et al. 2014), and metals are the most studied group of toxic substances (Mitchell et al. 2002; Sarma and Nandini 2006). Toxicity tests with these species are considered "acute", typically in 24–48 h of exposition to determine effective concentrations ($EC_{50}$), or "chronic", when conducted in longer periods, and used to evaluate its effect on the life cycle (e.g. reproduction, growth, offspring generation). Magalhães et al. (2014), performing acute tests, found that *C. dubia* was more sensitive for $Cu^{2+}$, $Cr^{3+}$, $Ni^{2+}$, $Zn^{2+}$ and *D. similis* for $Cr^{6+}$, $Cd^{2+}$, $Pb^{2+}$ and $Hg^{2+}$, and species had similar sensitivity to $Ag^+$ and $Cu^+$. This study also shown that *C. dubia* acute toxicity bioassay is a good tool to detect metal toxicity in mixtures under laboratory conditions. Chronic effects in laboratory exposition could be used to extrapolate the effect on the environment. Effects of metals on the reproduction of zooplankton include a decreased number of eggs, abortions, delayed age of first offspring and disruptions in ovarian development (Hook and Fisher 2001a, b; De Schamphelaere et al. 2004). Reproduction is a good bioindicator because target sites of metals in copepods and cladocerans include fat cells or oocytes (female sex cells) and may be influenced either by direct uptake of water or by accumulation via ingestion of contaminated food (Hook and Fisher 2001a). Fat cells, which are distributed throughout the digestive system, serve as sites for vitellogenin synthesis, which is a precursor of lipovitellin (Hook and Fisher 2001a, b). When exposed to metals, fat cells decrease the production of proteins in eggs, altering the nutrition of neonates during egg stage, leading to death of the embryo (Hook and Fisher 2001b). Metal pollution may strongly reduce abundance, species richness and diversity of zooplankton, and could lead to effects on trophic chains. In Sado River (Portugal), Monteiro et al. (1995) observed that only *Acanthocyclops robustus*, *Arcella vulgaris* and *Philodina* sp. tolerated high metal concentrations, and they reported a recovery in species composition and abundance on sites with lower metal pollution.

### Benthic macroinvertebrates

Benthic macroinvertebrates are insects, annelids, nematodes, some crustaceans and molluscs that are visible to the naked eye (>500 μm) and live in association with particles, substrates and sediments in aquatic environments. Members of this group are present in virtually all freshwater environments, are sedentary and have a relatively short life cycle (a few months to a few years), characteristics that make them ideal for the assessment of the ecological state of sites chronically or acutely affected by metals (Resh 2008).

Benthic macroinvertebrates are the most commonly used community to evaluate environmental condition in large-scale programs around the world (Buss et al. 2015). High concentrations of metals or long exposition to chronic concentrations can alter the composition of benthic macroinvertebrates community, reducing the abundance of sensitive organisms and influencing the drift of species (avoidance behaviour). These effects have been observed in microcosm studies (Clements 2004; Hickey and Golding 2002) and in situ (Clements and Kiffney 1994). The insect groups Ephemeroptera, Plecoptera and Trichoptera are reported as the most sensitive insects in Northern Hemisphere to indicate environments with high concentrations of metals (Clements et al. 1992; Gower et al. 1994; Winner et al. 1980), whereas dipteran insects (Chironomidae) (Gower et al. 1994), flatworms (Gower et al. 1994) and annelid oligochaete of the family Lumbriculidae (Santoro et al. 2009) are reported as the most resistant.

The potential for bioaccumulation of metals by benthic macroinvertebrates is directly related to the concentration of metals in the sediment. Animals that feed on deposited material bioaccumulate more metals than predators and filter-feeders (organisms that feed on suspended material) (Eyong 2008; Santoro et al. 2009). Enk and Mathis (1977) found $Cd^{2+}$ concentrations five and ten times higher in aquatic insects than the one found in sediment and water, respectively. Farag et al. (1998) observed that although

metals are available for biotransference, they do not bio-magnify, and studies with $Zn^{2+}$, $Cu^{2+}$, $Pb^{2+}$ and $Cd^{2+}$ showed a direct relationship between the concentration of these metals in sediment and in water with the bioaccumulation by benthic macroinvertebrates, but no relationship with trophic levels was observed (Goodyear and McNeill 1999; Memmert 1987).

### Fish

Advantages of using this group for biomonitoring are the possibility to extract and analyse isolated organs and biomarkers such as delta-aminolevulinic acid dehydratase, metallothionein, glutathione S-transferase among others can be used (Olsvik et al. 2001), and also because of their relative long life cycle that allows tracking changes over time (Resh 2008).

Paquin et al. (2002) divided the physiological mechanism of metal toxicity in fish into three categories: (1) monovalent metals such as $Ag^+$ and $Cu^+$ affect the transport of $Na^+$ and $Cl^-$. In the case of copper, although its prevalence in the environment is in divalent form ($Cu^{2+}$), it is reduced to $Cu^+$ before passing through biological membrane. The consequence of osmoregulatory dysfunction is a redistribution of ions and water between the internal fluids of fish, resulting in a decrease in $Na^+$, $Cl^-$ and other ions in plasma, which in turn triggers a sequence of events culminating in a cardiovascular collapse and death; (2) divalent metals such as $Cd^{2+}$ and $Zn^{2+}$ disrupt the metabolism of $Ca^{2+}$, triggering a hypocalcemia (as described above); and (3) metals such as $Pb^{2+}$ and $Hg^{2+}$ cross the gills and act in the central nervous system.

Generally, gills are the first organ of contact with metals, through the respiration of dissolved parts. In general, lower concentrations of metals have ion regulatory effects in gills of freshwater fish, whereas higher concentrations cause gill damage and mucus accumulation, triggering respiratory effects (Playle 1998). In addition to the use of biomarkers and the observation of gill damage, the concentration of metal bioaccumulated also provided a tool for biomonitoring. Routes of accumulation are important for monitoring the environment and for estimating hazard risk of exposure to aquatic biota and humans. According to the routes of assimilation (in water or through feeding), metal will bioaccumulate in fish in different organs and bioaccumulation will depend on metal species. In general, metal levels in the liver represent metal storage from water and sediments where the fish species live (Karadede et al. 2004; Jezierska and Witeska 2006) and are recommended as a general environmental indicator of external medium pollution more than other fish organs—except for Pb that is only directly absorbed through water and accumulates preferentially in gills (Čelechovská et al. 2007; Has-Schön et al. 2008; Tepe et al. 2008; Wei et al. 2014).

Independently of the route of exposition, organs have more affinity for some specific metal species accumulation. Generally, liver is the primary organ for Cu and Ag accumulation, kidney for Cd and Zn accumulation, gills for Ni and Pb accumulation (Čelechovská et al. 2007; Has-Schön et al. 2008; Tepe et al. 2008; Wei et al. 2014, Yamazaki et al. 1996), and spleen and gallbladder for Cr accumulation (Franklin et al. 2005). Compared with the gill, kidney and liver, muscle typically contains low metal concentrations (Alcorlo et al. 2006; Wei et al. 2014), although Hg can accumulate more easily in muscle than in other organs (Čelechovská et al. 2007; Has-Schön et al. 2008). $Hg^{2+}$ ingestion may also lead to accumulation in the form of methyl-Hg. Although fish are incapable of methylation, bacteria in their digestive tract can transform $Hg^{2+}$ into methyl-Hg (Rudd et al. 1980). Methyl-Hg (absorbed through water and food) accumulates preferentially in gills, muscles and brain (Phillips and Gregory 1979; Phillips and Buhler 1980; Turner and Swick 1983; Wei et al. 2014). The persistence of methyl-Hg in fish is relatively high due to its slow metabolism. The half-life varies among species, usually ranging from one to 3 years (Bisinoti and Jardim 2004).

### Conclusion

Understanding all mechanisms that influence toxicity of metals in aquatic systems is a difficult task due to the influence of chemical, physical and biological factors. Metal speciation greatly determines the behaviour and toxicity of metals in the environment, but there are exceptions that make biomonitoring a necessary approach to evaluate the effect on the biota. The evaluation of biological responses is an effective approach in identifying metal-induced damages in aquatic environments because it integrates the influence of environmental parameters on metals and their effects. The choice of the best biological response to detect metal toxicity effect depends on the type of exposure (short term or long term), metals of concern, routes of exposition and target organisms. Laboratory ecotoxicity bioassays could be used to infer the effect of metals in the environment. Also, some mathematical models have been developed to predict metal toxicity, but they have some limitations because there is a lack of information about chronic toxicity of metal mixtures and also there is still a need to understand how metals interact between themselves and with organisms. The development of more robust toxicity prediction models will allow to estimate the potential effect of metal mixtures, but to have an adequate assessment of the impact of those stressors, information from ecotoxicological tests, in situ biomonitoring and/or metabolic assessment are necessary.

# References

Ahner BA, Lee JG, Price NM, Morel FMM (1998) Phytochelatin concentrations in the equatorial Pacific. Deep Sea Res Part I 45:1779–1796

Alcedo JF, Wetterhahn KE (1990) Chromium toxicity and carcinogenesis. Int Rev Exp Pathol 31:85

Alcorlo P, Otero M, Crehuet M, Baltanas A, Montes C (2006) The use of the red swamp crayfish (Procambarus clarkii, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the river Guadiamar (SW, Spain). Sci Total Environ 366:380–390

Allen HE, Janssen CR (2006) Incorporating bioavailability into criteria for metals. In: Twardowska I, Allen HE, Häggblom MM, Stefaniak S (eds) Soil and water pollution monitoring, protection and remediation. Springer, Netherlands, pp 93–105

Allen HE, Fu G, Deng B (1993) Analysis of acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. Environ Toxicol Chem 12:1441–1453

Al-Rub FAA, El-Naas MH, Benyahia F, Ashour I (2004) Biosorption of nickel on blank alginate beads, free and immobilized algal cells. Process Biochem 39:1767–1773

Al-Rub FAA, El-Naas MH, Ashour I, Al-Marzouqi M (2006) Biosorption of copper on Chlorella vulgaris from single, binary and ternary metal aqueous solutions. Process Biochem 41:457–464

Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS (2006) Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat Toxicol 76:160–202

ANZECC, Australian and New Zealand Environment and Conservation Council and ARMCANZ, Agriculture and Resource Management Council of Australia and New Zealand (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Volume 2. Aquatic Ecosystems—Rationale and Background Information. Australian Water Association, Artarmon, New South Wales, Australia; New Zealand Water and Wastes Association, Onehunga, Auckland, New Zealand

Atli G, Canli M (2010) Response of antioxidant system of freshwater fish Oreochromis niloticus to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotoxicol Environ Saf 73:1884–1889

Backhaus T, Faust M (2012) Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ Sci Technol 46:2564–2573

Baer KN, Thomas P (1990) Influence of capture stress, salinity and reproductive status on zinc associated with metallothionein like-proteins in the liver of three teleost species. Mar Environ Res 29:277–287

Baken S, Degryse F, Verheyen L, Merckx R, Smolders E (2011) Metal complexation properties of freshwater dissolved organic matter are explained by its aromaticity and by anthropogenic ligands. Environ Sci Technol 45:2584–2590

Berry WJ, Hansen DJ, Boothman WS, Mahony JD, Robson DL, Di Toro DM, Corbin JM (1996) Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. Environ Toxicol Chem 15:2067–2079

Bezerra PSS, Takiyama LR, Bezerra CWB (2009) Complexation of metal ions by dissolved organic matter: modeling and application to real systems. Acta Amazon 39:639–648

Bisinoti MC, Jardim WF (2004) Behavior of methylmercury in the environment. Quim Nova 27:593–600

Bjerregaard P, Andersen O (2007) Ecotoxicology of metals: sources, transport, and effects in the ecosystem. In: Gunnar FN, Bruce AF, Monica N, Lars TF (eds) Handbook on the toxicology of metals, 3rd edn. Academic Press, Burlington, pp 251–277

Blackmore G, Wang WX (2002) Uptake and efflux of Cd and Zn by the green mussel Perna viridis after metal preexposure. Environ Sci Technol 36:989–995

Borgmann U, Norwood WP, Dixon DG (2004) Re-evaluation of metal bioaccumulation and chronic toxicity in Hyalella azteca using saturation curves and the biotic ligand model. Environ Pollut 13:469–484

Brauner CJ, Wood C (2002a) Ionoregulatory development and the effect of chronic silver exposure on growth, survival, and sublethal indicators of toxicity in early life stages of rainbow trout (Oncorhynchus mykiss). J Comp Physiol B 172:153–162

Brauner CJ, Wood CM (2002b) Effect of long-term silver exposure on survival and ionoregulatory development in rainbow trout (Oncorhynchus mykiss) embryos and larvae, in the presence and absence of added dissolved organic matter. Comp Biochem Physiol C 133:161–173

Brauner CJ, Wilson J, Kamunde C, Wood CM (2003) Water chloride provides partial protection during chronic exposure to waterborne silver in rainbow trout (Oncorhynchus mykiss) embryos and larvae. Physiol Biochem Zool 76:803–815

Buss DF, Carlise DM, Chon TS, Culp J, Harding JS et al (2015) Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. Environ Monit Assess. DOI 10.1007/s10661-014-4132-8

Butler BA, Ranville JF, Ross PE (2008) Direct versus indirect determination of suspended sediment associated metals in a mining-influenced watershed. Appl Geochem 23:1218–1231

Campbell PGC (1995) Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier A, Turner DR (eds) Metal speciation and bioavailability in aquatic systems. Wiley, Chichester, pp 45–102

Carney Almroth B, Albertsson E, Sturve J, Förlin L (2008) Oxidative stress, evident in antioxidant defences and damage products, in rainbow trout caged outside a sewage treatment plant. Ecotoxicol Environ Saf 70:370–378

CCME, Canadian Council of Ministers of the Environment (2007) A protocol for the derivation of water quality guidelines for the protection of aquatic life. Canadian Council of Ministers of the Environment, Winnipeg

CCME, Canadian Council of Ministers of the Environment (2012) Metal mining environmental effects monitoring (EEM) technical guidance document. Canadian Council of Ministers of the Environment, Winnipeg

Čelechovská O, Svobodová Z, Žlábek V, Macharáčková B (2007) Distribution of metals in tissues of the common carp (Cyprinus carpio L.). Acta Vet Brno 76:93–100

Cervantes C, Campos-García J, Devars S, Gutiérrez-Corona F, Loza-Tavera H, Torres-Guzmán JC et al (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 25:335–347

Chen ZZ, Zhu L, Yao K, Wang XJ, Ding JN (2009) Interaction between calcium and lead affects to embryo of zebrafish (Danio rerio). Huan Jing Ke Xue 30:1205–1209

Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475–486

Clements WH (2004) Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. Ecol Appl 14:954–967

Clements WH, Kiffney PM (1994) Assessing contaminant effects at higher levels of biological organization. Environ Toxicol Chem 13:357–359

Clements WH, Cherry DS, Hassel JHV (1992) Assessment of the impact of heavy metals on benthic communities at the Clinch River (Virginia): evaluation of an index of community sensitivity. Can J Fish Aquat Sci 49:1686–1694

Cornelis R, Nordberg M (2007) General chemistry, sampling, analytical methods, and speciation. In. In: Gunnar FN, Bruce AF, Monica N, Lars TF (eds) Handbook on the toxicology of metals, 3rd edn. Academic Press, Burlington, pp 197–208

Da Cruz ACS, Couto BC, Nascimento IA et al (2007) Estimation of the critical effect level for pollution prevention based on oyster embryonic development toxicity test: the search for reliability. Environ Int 33:589–595

De Schamphelaere KAC, Janssen CR (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 KAC, Canli M, Van Lierde V et al (2004) Reproductive toxicity of dietary zinc to Daphnia magna. Aquat Toxicol 70:233–244

De Schamphelaere KA, Lofts S, Janssen CR (2005) Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters. Environ Toxicol Chem 24:1190–1197

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

Duong TT, Morin S, Herlory O, Feurtet-Mazel A, Coste M, Boudou A (2008) Seasonal effects of cadmium accumulation in periphytic diatom communities of freshwater biofilms. Aquat Toxicol 90:19–28

El-Enany AE, Issa AA (2001) Proline alleviates heavy metal stress in Scenedesmus armatus. Folia Microbiol 46:227–230

English TE, Storey KB (2003) Freezing and anoxia stresses induce expression of metallothionein in the foot muscle and hepatopancreas of the marine gastropod Littorina littorea. J Exp Biol 206:2517–2524

Enk M, Mathis BJ (1977) Distribution of cadmium and lead in a stream ecosystem. Hydrobiologia 52:153–158

Eroglu A, Dogan Z, Kanak EG, Atli G, Canli M (2014) Effects of heavy metals (Cd, Cu, Cr, Pb, Zn) on fish glutathione metabolism. Environ Sci Pollut Res 21:1–9

Escher BI, Hermens JLM (2004) Peer reviewed: internal exposure: linking bioavailability to effects. Environ Sci Technol 38:455A–462A

Eyong BE (2008) Distribution of arsenic and other heavy metals in sediments and their effects on benthic macroinvertebrates in the Gallinas river. Dissertation, New Mexico Highlands University San Miguel County

Farag AM, Woodward DF, Goldstein JN, Brumbaugh W, Meyer JS (1998) Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. Arch Environ Contam Toxicol 34:119–127

Forsberg J, Dahlqvist R, Gelting-Nystro J, Ingri J (2006) Trace metal speciation in brackish water using diffusive gradients in thin films and ultrafiltration: comparison of techniques. Environ Sci Technol 40:3901–3905

Franklin NM, Stauber JL, Lim RP, Petocz P (2002) Toxicity of metal mixtures to a tropical freshwater alga (Chlorella sp.): the effect of interactions between copper, cadmium, and zinc on metal cell binding and uptake. Environ Toxicol Chem 21:2412–2422

Franklin NM, Glover CN, Nicol JA, Wood CM (2005) Calcium/cadmium interactions at uptake surfaces in rainbow trout: waterborne versus dietary routes of exposure. Environ Toxicol Chem 24:2954–2964

Funes V, Alhama J, Navas JI, López-Barea J, Peinado J (2006) Ecotoxicological effects of metal pollution in two mollusc species from the Spanish South Atlantic littoral. Environ Pollut 139:214–223

Gasparatos D (2013) Sequestration of heavy metals from soil with Fe–Mn concretions and nodules. Environ Chem Lett 11:1–9

Gerhardt A (2000) Biomonitoring of polluted water. Trans Tech Publications Ltd.

Gonçalves J, Nicoloso F, Becker A, Pereira L et al (2009) Photosynthetic pigments content, δ-aminolevulinic acid dehydratase and acid phosphatase activities and mineral nutrients concentration in cadmium-exposed Cucumis sativus L. Biologia 64:310–318

Goodyear KL, McNeill S (1999) Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. Sci Total Environ 229:1–19

Gower AM, Myers G, Kent M, Foulkes ME (1994) Relationships between macroinvertebrate communities and environmental variables in metal-contaminated streams in south-west England. Freshw Biol 32:199–221

Grosell M, Gerdes R, Brix KV (2006) Influence of Ca, humic acid and pH on lead accumulation and toxicity in the fathead minnow during prolonged water-borne lead exposure. Comp Biochem Physiol C 143:473–483

Gunkel-Grillon P, Laporte-Magoni C, Lemestre M, Bazire N (2014) Toxic chromium release from nickel mining sediments in surface waters, New Caledonia. Environ Chem Lett 12:511–516

Hagger JA, Jones MB, Leonard DR, Owen R, Galloway TS (2006) Biomarkers and integrated environmental risk assessment: are there more questions than answers? Integr Environ Assess Manage 2:312–329

Han J, Ma D, Quan X, Wang J, Yan Q (2005) Bioavailability of zinc in the sediment to the estuarine amphipod Grandidierella japonica. Hydrobiologia 541:149–154

Has-Schön E, Bogut I, Kralik G, Bogut S, Horvatić J, Čačić I (2008) Heavy metal concentration in fish tissues inhabiting waters of "Buško Blato" reservoir (Bosna and Herzegovina). Environ Monit Assess 144:15–22

Heijerick DG, Janssen CR, De Coen WM (2003) The combined effects of hardness, pH, and dissolved organic carbon on the chronic toxicity of Zn to D. magna: development of a surface response model. Arcah Environ Contam Toxicol 44:0210–0217

Hellou J, Ross NW, Moon TW (2012) Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environ Sci Pollut Res 19:2007–2023

Henderson R, Hobbie J, Landrigan P, Mattisoti D, Perera F, Pfttaer E et al (1987) Biological markers in environmental health research. Environ Health Perspect 7:3–9

Hering JG (2009) Metal speciation and bioavailability: revisiting the 'big questions'. Environ Chem 6:290–293

Hickey CW, Golding LA (2002) Response of macroinvertebrates to copper and zinc in a stream mesocosm. Environ Toxicol Chem 21:1854–1863

Ho JW (1990) Micro assay for urinary δ-aminolevulinic acid and porpholilinogen by high-performance liquid chromatography with pre-column derivatization. J Chromatogr B 527:134–139

Hook SE, Fisher NS (2001a) Sublethal effects of silver in zooplankton: importance of exposure pathways and implications for toxicity testing. Environ Toxicol Chem 20:568–574

Hook SE, Fisher NS (2001b) Reproductive toxicity of metals in calanoid copepods. Mar Biol 138:1131–1140

Iwasaki Y, Kamo M, Naito W (2014) Testing an application of a biotic ligand model to predict acute toxicity of metal mixtures to rainbow trout. Environ Toxicol Chem. doi:10.1002/etc.2780

Jezierska B, Witeska M (2006) The metal uptake and accumulation infish living in polluted water. In: Twardowska I, Allen HE, Häggblom MM, Stefaniak S (eds) Soil and water pollution monitoring, protection and remediation. Springer, Netherlands, pp 107–114

Jho EH, An J, Nam K (2011) Extended biotic ligand model for prediction of mixture toxicity of Cd and Pb using single metal toxicity data. Environ Toxicol Chem 30:1697–1703

Kägi JHR (1993) Evolution, structure and chemical activity of class I metallothioneins: an overview. In: Suzuki KT, Imura N, Kimura M (eds) Metallothioneins III—biological roles and medical implications. Birkhäuser, Basel, pp 29–55

Kamo M, Nagai T (2008) An application of the biotic ligand model to predict the toxic effects of metal mixtures. Environ Toxicol Chem 27:1479–1487

Karadede H, Oymak SA, Ünlü E (2004) Heavy metals in mullet, Liza abu, and catfish, Siluris triostegus, from the Atatürk Dam Lake (Euphrates), Turkey. Environ Int 30:183–188

Keteles KA, Fleeger JW (2001) The contribution of Ecdysis to the fate of copper, zinc and cadmium in grass shrimp, Palaemonetes pugio Holthius. Mar Pollut Bull 42:1397–1402

Klinck J, Dunbar M, Brown S, Nichols J, Winter A, Hughes C, Playle RC (2005) Influence of water chemistry and natural organic matter on active and passive uptake of inorganic mercury by gills of rainbow trout (Oncorhynchus mykiss). Aquat Toxicol 72:161–175

Komjarova I, Blust R (2009a) Application of a stable isotope technique to determine the simultaneous uptake of cadmium, copper, nickel, lead, and zinc by the water flea Daphnia magna from water and the green algae Pseudokirchneriella subcapitata. Environ Toxicol Chem 28:1739–1748

Komjarova I, Blust R (2009b) Effect of Na, Ca and pH on simultaneous uptake of Cd, Cu, Ni, Pb, and Zn in the water flea Daphnia magna measured using stable isotopes. Aquat Toxicol 94:81–86

Komjarova I, Blust R (2009c) Effects of Na, Ca, and pH on the simultaneous uptake of Cd, Cu, Ni, Pb and Zn in the zebrafish Danio rerio: a stable isotope experiment. Environ Sci Technol 43:7958–7963

Kosolapov DB, Kuschk P, Vainshtein MB, Vatsourina AV, Wiessner A, Kästner M, Müller RA (2004) Microbial processes of heavy metal removal from carbon-deficient effluents in constructed wetlands. Eng Life Sci 4:403–411

Koukal B, Rosse P, Reinhardt A, Ferrari B, Wilkinson KJ, Loizeau JL, Dominik J (2007) Effect of Pseudokirchneriella subcapitata (Chlorophyceae) exudates on metal toxicity and colloid aggregation. Water Res 41:63–70

Kozlova T, Wood CM, McGeer JC (2009) The effect of water chemistry on the acute toxicity of nickel to the cladoceran Daphnia pulex and the development of a biotic ligand model. Aquat Toxicol 91:221–228

Kurek E, Francis AJ, Bollag JM (1991) Immobilization of cadmium by microbial extracellular products. Arch Environ Contam Toxicol 21:106–111

Laurie AD (2004) Quantitation of metallothionein mRNA from the New Zealand common bully (Gobiomorphus cotidianus) and its implications for biomonitoring. New Zeal J Mar Freshw 38:869–877

Lavoie I, Lavoie M, Fortin C (2012) A mine of information: benthic algal communities as biomonitors of metal contamination from abandoned tailings. Sci Total Environ 425:231–241

Lee JG, Ahner BA, Morel FMM (1996) Export of cadmium and phytochelatin by the marine diatom Thalassiosira weissflogii. Environ Sci Technol 30:1814–1821

Lemaire S, Keryer E, Stein M, Schepens II et al (1999) Heavy-metal regulation of thioredoxin gene expression in Chlamydomonas reinhardtii. Plant Physiol 120:773–778

Livingstone DR (2001) Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar Pollut Bull 42:656–666

Lock K, Janssen CR (2002) Mixture toxicity of zinc, cadmium, copper, and lead to the potworm Enchytraeus albidus. Ecotoxicol Environ Safe 52:1–7

Lombardi PE, Peri SI, Verrengia Guerrero NR (2010) ALA-D and ALA-D reactivated as biomarkers of lead contamination in the fish Prochilodus lineatus. Ecotoxicol Environ Saf 73:1704–1711

Macfie SM, Welbourn PM (2000) The cell wall as a barrier to uptake of metal ions in the unicellular green alga Chlamydomonas reinhardtii (Chlorophyceae). Arch Environ Contam Toxicol 39:413–419

Machado AADS, Hoff MLM, Klein RD, Cordeiro GJ et al (2014) Oxidative stress and DNA damage responses to phenanthrene exposure in the estuarine guppy Poecilia vivipara. Mar Environ Res 98:96–105

Magalhães DP, Marques MRC, Baptista DF, Buss DF (2014) Selecting a sensitive battery of bioassays to detect toxic effects of metals in effluents. Ecotoxicol Environ Safe 110:73–81

Marasinghe Wadige CP, Taylor AM, Maher WA, Ubrihien RP, Krikowa F (2014) Effects of lead-spiked sediments on freshwater bivalve, Hyridella australis: linking organism metal exposure-dose-response. Aquat Toxicol 149:83–93

Marcussen H, Dalsgaard A, Holm PE (2008) Content, distribution and fate of 33 elements in sediments of rivers receiving wastewater in Hanoi Vietnam. Environ pollut 155:41–51

Matagi SV, Swai D, Mugabe R (1998) A review of heavy metal removal mechanisms in wetlands. Afr J Trop Hydrobiol Fish 8:13–25

McKnight DM, Morel FMM (1979) Release of weak and strong copper-complexing agents by algae. Limnol Oceanogr 24:823–837

McWilliams PG, Potts WTW (1978) The effects of pH and calcium concentrations on gill potentials in the brown trout, Salmo trutta. J Comp Physiol 126:277–286

Memmert U (1987) Bioaccumulation of zinc in two freshwater organisms (Daphnia magna, crustacea and Brachydanio rerio, pisces). Water Res 21:99–106

Mitchell EJ, Burgess J, Stuetz R (2002) Developments in ecotoxicity testing. Rev Environ Sci Biotechnol 1:169–198

Monteiro MT, Oliveira R, Vale C (1995) Metal stress on the plankton communities of Sado River (Portugal). Water Res 29:695–701

Monteiro DA, Rantin FT, Kalinin AL (2010) Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, Brycon amazonicus (Spix and Agassiz, 1829). Ecotoxicol 19:105–123

Mosleh YY, Paris-Palacios S, Arnoult F, Couderchet M, Biagianti-Risbourg S, Vernet G (2004) Metallothionein induction in aquatic oligochaete Tubifex tubifex exposed to herbicide isoproturon. Environ Toxicol 19:88–93

Mu Y, Wu F, Chen C, Liu Y, Zhao X, Liao H, Giesy JP (2014) Predicting criteria continuous concentrations of 34 metals or metalloids by use of quantitative ion character-activity relationships–species sensitivity distributions (QICAR–SSD) model. Environ Pollut 188:50–55

Munger C, Hare L (1997) Relative importance of water and food as cadmium sources to an aquatic insect (Chaoborus punctipennis): implications for predicting Cd bioaccumulation in nature. Environ Sci Technol 31:891–895

Munné A, Tirapu L, Sola C, Olivella L, Vilanova M, Ginebreda A, Prat N (2012) Comparing chemical and ecological status in catalan rivers: analysis of river quality status following the water framework directive. Emerging and priority pollutants in rivers. Springer, Berlin, pp 243–265

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216

Nishikawa K, Yamakoshi Y, Uemura I, Tominaga N (2003) Ultrastructural changes in Chlamydomonas acidophila (Chlorophyta) induced by heavy metals and polyphosphate metabolism. FEMS Microbiol Ecol 44:253–259

Niyogi S, Wood CM (2004) Biotic ligand model, a flexible tool for developing sitespecific water quality guidelines for metals. Environ Sci Technol 38:6177–6192

Niyogi S, Kent R, Wood CM (2008) Effects of water chemistry variables on gill binding and acute toxicity of cadmium in rainbow trout (Oncorhynchus mykiss): a biotic ligand model (BLM) approach. Comp Biochem Physiol C 148:305–314

Norwood WP, Borgmann U, Dixon DG, Wallace A (2003) Effects of metal mixtures on aquatic biota: a review of observations and methods. Hum Ecol Risk Assess 9:795–811

Nunes B, Capela RC, Sérgio T, Caldeira C, Gonçalves F, Correia AT (2014) Effects of chronic exposure to lead, copper, zinc, and cadmium on biomarkers of the European eel, Anguilla anguilla. Environ Sci Pollut Res 21:5689–5700

Nystrand MI, Österholm P, Nyberg ME, Gustafsson JP (2012) Metal speciation in rivers affected by enhanced soil erosion and acidity. Appl Geochem 27:906–916

Olsvik PA, Hindar K, Zachariassen KE, Andersen RA (2001) Brown trout (Salmo trutta) metallothioneins as biomarkers for metal exposure in two Norwegian rivers. Biomarkers 6:274–288

Ospina-Alvarez N, Głaz Ł, Dmowski K, Krasnodębska-Ostręga B (2014) Mobility of toxic elements in carbonate sediments from a mining area in Poland. Environ Chem Lett 12:435–441

Oueslati W, Added A, Abdeljaoued S (2010) Vertical profiles of simultaneously extracted metals (SEM) and acid-volatile sulfide in a changed sedimentary environment: Ghar El Melh Lagoon, Tunisia. Soil Sediment Contam 19:696–706

Oven M, Grill E, Golan-Goldhirsh A, Kutchan TM, Zenk MH (2002) Increase of free cysteine and citric acid in plant cells exposed to cobalt ions. Phytochem 60:467–474

Pandey LK, Kumar D, Yadav A, Rai J, Gaur JP (2014) Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. Ecol Indic 36:272–279

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PG et al (2002) The biotic ligand model: a historical overview. Comp Biochem Physiol C Toxicol Pharmacol 133:3–35

Pesavento M, Alberti G, Biesuz R (2009) Analytical methods for determination of free metal ion concentration, labile species fraction and metal complexation capacity of environmental waters: a review. Anal Chim Acta 631:129–141

Phillips GR, Buhler DR (1980) Mercury accumulation in and growth rate of rainbow trout, Salmo gairdneri, stocked in an eastern Oregon reservoir. Arch Environ Contam Toxicol 9:99–107

Phillips GR, Gregory RW (1979) Assimilation efficiency of dietary methylmercury by northern pike (Esox lucius). J Fish Res Board Can 36:1516–1519

Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P (2003) Heavy metal-induced oxidative stress in algae. J Phycol 39:1008–1018

Playle RC (1998) Modelling metal interactions at fish gills. Sci Total Environ 219:147–163

Playle RC (2004) Using multiple metal–gill binding models and the toxic unit concept to help reconcile multiple-metal toxicity results. Aquat Toxicol 67:359–370

Prica M, Dalmacija B, Rončević S, Krčmar D, Bečelić M (2008) A comparison of sediment quality results with acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) ratio in Vojvodina (Serbia) sediments. Sci Total Environ 389:235–244

Raftopoulou EK, Dailianis S, Dimitriadis VK, Kaloyianni M (2006) Introduction of cAMP and establishment of neutral lipids alterations as pollution biomarkers using the mussel Mytilus

galloprovincialis. Correlation with a battery of biomarkers. Sci Total Environ 368:597–614

Rainbow PS, Luoma SN (2011) Metal toxicity, uptake and bioaccumulation in aquatic invertebrates—modelling zinc in crustaceans. Aquat Toxicol 105:455–465

Reeve R (2002) Introduction to environmental analysis. Wiley, UK

Resh VH (2008) Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environ Monit Assess 138:131–138

Richards JG, Burnison BK, Playle RC (1999) Natural and commercial dissolved organic matter protects against the physiological effects of a combined cadmium and copper exposure on rainbow trout (Oncorhynchus mykiss). Can J Fish Aquat Sci 56:407–418

Robinson NJ, Tommey AM, Kuske C, Jackson PJ (1993) Plant metallothioneins. Biochem J 295:1–10

Robinson KA, Baird DJ, Wrona FJ (2003) Surface metal adsorption on zooplankton carapaces: implications for exposure and effects in consumer organisms. Environ Pollut 122:159–167

Rocha JC, Junior ES, Zara LF, Rosa AH, Dos Santos A, Burba P (2000) Reduction of mercury (II) by tropical river humic substances (Rio Negro)—a possible process of the mercury cycle in Brazil. Talanta 53:551–559

Rocha JC, Sargentini E Jr, Zara LF, Rosa AH, Dos Santos A, Burba P (2003) Reduction of mercury (II) by tropical river humic substances (Rio Negro)—Part II. Influence of structural features (molecular size, aromaticity, phenolic groups, organically bound sulfur). Talanta 61:699–707

Rodea-Palomares I, González-García C, Leganés F, Fernández-Piñas F (2009) Effect of pH, EDTA, and anions on heavy metal toxicity toward a bioluminescent cyanobacterial bioreporter. Arch Environ Contam Tox 57:477–487

Rodrigues ALS, Rocha JBT, Pereira ME, Souza DO (1996) δ-aminolevulinic acid dehydratase activity in weanling and adult rats exposed to lead acetate. Bull Environ Contam Toxicol 57:47–53

Rosa AH, Rocha JC, Burba P (2002) Extraction and exchange behavior of metal species in therapeutically applied peat. Talanta 58:969–978

Rudd JW, Furutani A, Turner MA (1980) Mercury methylation by fish intestinal contents. Appl Environ Microbiol 40:777–782

Saglam D, Atli G, Canli M (2013) Investigations on the osmoregulation of freshwater fish (Oreochromis niloticus) following exposures to metals (Cd, Cu) in differing hardness. Ecotoxicol Environ Saf 92:79–86

Sánchez-Marín P, Lorenzo JI, Blust R, Beiras R (2007) Humic acids increase dissolved lead bioavailability for marine invertebrates. Environ Sci Technol 41:5679–5684

Santore RC, Di Toro DM, Paquin PR, Allen HE, Meyer JS (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

Santoro A, Blo G, Mastrolitti S, Fagioli F (2009) Bioaccumulation of heavy metals by aquatic macroinvertebrates along the Basento River in the South of Italy. Water Air Soil Pollut 201:19–31

Sarin C, Hall JM, Cotter-Howells J, Killham K, Cresser MS (2000) Influence of complexation with chloride on the responses of a lux-marked bacteria bioassay to cadmium, copper, lead, and mercury. Environ Toxicol Chem 19:259–264

Sarma SSS, Nandini S (2006) Review of recent ecotoxicological studies on cladocerans. J Environ Sci Health B 41:1417–1430

Schamphelaere De, Karel AC, Janssen CR (2004) Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (Oncorhynchus mykiss): comparison with other fish species and development of a biotic ligand model. Environ Sci Technol 38:6201–6209

Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker PM (2002) The role of phytochelatins in constitutive and adaptive

heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. J Exp Bot 53:2381–2392

Schmidt TS, Clements WH, Mitchell KA, Church SE, Wanty RB, Fey DL et al (2010) Development of a new toxic-unit model for the bioassessment of metals in streams. Environ Toxicol Chem 29:2432–2442

Schubauer-Berigan MK, Dierkes JR, Monson PD, Ankley GT (1993) pH-Dependent toxicity of Cd, Cu, Ni, Pb and Zn to Ceriodaphnia dubia, Pimephales promelas, Hyalella azteca and Lumbriculus variegatus. Environ Toxicol Chem 12:1261–1266

Schwartz ML, Curtis PJ, Playle RC (2004) Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem 23:2889–2899

Sevcikova M, Modra H, Slaninova A, Svobodova Z (2011) Metals as a cause of oxidative stress in fish: a review. Vet Med 56:537–546

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753

Sharma SS, Schat H, Vooijs R, Van Heerwaarden LM (1999) Combination toxicology of copper, zinc, and cadmium in binary mixtures: concentration-dependent antagonistic, nonadditive, and synergistic effects on root growth in Silene vulgaris. Environ Toxicol Chem 18:348–355

Singh HP, Mahajan P, Kaur S, Batish DR, Kohli RK (2013) Chromium toxicity and tolerance in plants. Environ Chem Lett 11:229–254

Sinha S, Bhatt K, Pandey K, Singh S, Saxena R (2003) Interactive metal accumulation and its toxic effects under repeated exposure in submerged plant najas indica cham. Bull Environ Contam Toxicol 70:0696–0704

Siripornadulsil S, Traina S, Verma DPS, Sayre RT (2002) Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. Plant Cell Online 14:2837–2847

Smiejan A, Wilkinson KJ, Rossier C (2003) Cd bioaccumulation by a freshwater bacterium, Rhodospirillum rubrum. Environ Sci Technol 37:701–706

Smith SR (2009) A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environ Int 35:142–156

Sofyan A, Shaw JR, Birge WJ (2006) Metal trophic transfer from algae to cladocerans and the relative importance of dietary metal exposure. Environ Toxicol Chem 25:1034–1041

Stankovic S, Kalaba P, Stankovic AR (2014) Biota as toxic metal indicators. Environ Chem Lett 12:63–84

Stockdale A, Tipping E, Lofts S, Fott J et al (2014) Metal and proton toxicity to lake zooplankton: a chemical speciation based modelling approach. Environ Pollut 186:115–125

Stockdale A, Tipping E, Lofts S, Ormerod SJ, Clements WH, Blust R (2010) Toxicity of proton–metal mixtures in the field: linking stream macroinvertebrate species diversity to chemical speciation and bioavailability. Aquat Toxicol 100:112–119

Tamas MJ, Wysocki R (2001) Mechanisms involved in metalloid transport and tolerance acquisition. Curr Genet 40:2–12

Templeton DM, Cherian MG (1991) Toxicological significance of metallothionein. Meth Enzymol 205:11–24

Templeton DM, Ariese F, Cornelis R, Danielsson L-G, Muntau H, Leeuwen HPV et al (2000) Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches. Pure Appl Chem 72:1453–1470

Tepe Y, Türkmen M, Türkmen A (2008) Assessment of heavy metals in two commercial fish species of four Turkish seas. Environ Monit Assess 146:277–284

Tipping E (1998) Humic Ion-Binding Model VI: an improved description of the interactions of protons and metal ions with humic substances. Aquat Geochem 4:3–47

Tipping E, Lofts S, Sonke JE (2011) Humic Ion-Binding Model VII: a revised parameterisation of cation-binding by humic substances. Environ Chem 8:225–235

Tsiridis V, Petala M, Samaras P, Hadjispyrou S, Sakellaropoulos G, Kungolos A (2006) Interactive toxic effects of heavy metals and humic acids on Vibrio fischeri. Ecotoxi Environ Saf 63:158–167

Tsui MTK, Wang WX (2006) Acute toxicity of mercury to Daphnia magna under different conditions. Environ Sci Technol 40:4025–4030

Turner MA, Swick AL (1983) The English-Wabigoon River System: IV. Interaction between mercury and selenium accumulated from waterborne and dietary sources by northern pike (Esox lucius). Can J Fish Aquat Sci 40:2241–2250

USEPA, United States Environmental Protection Agency (1995) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish, 2nd edn. Office of Water, Washington, DC: EPA 841-B-99-002

USEPA, United States Environmental Protection Agency (2007) Framework for metals risk assessment. Office of the Science Advisor, Risk Assessment Forum. Washington, DC: EPA/120/R-07/001

USEPA, United States Environmental Protection Agency (2009) National recommended water quality criteria. Office of Water, Office of Science and Technology, Washington

Velma V, Tchounwou PB (2010) Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish Carassius auratus. Mutat Res 698:43–51

Vignati DA, Loizeau J-L, Rossé P, Dominik J (2006) Comparative performance of membrane filters vs. high-surface area filtration cartridges for the determination of element concentrations in freshwater systems. Water Res 40:917–924

Vignati DA, Dominik J, Beye ML, Pettine M, Ferrari BJD (2010) Chromium (VI) is more toxic than chromium (III) to freshwater algae: a paradigm to revise? Ecotoxicol Environ Saf 73:743–749

Vijver MG, van Gestel CAM, Lanno RP, van Straalen NM, Peijnenburg WJGM (2004) Internal metal sequestration and its ecotoxicological relevance: a review. Environ Sci Technol 38:4705–4712

Wallace WG, Lopez GR (1996) Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuaries 19:923–930

Wallace WG, Luoma SN (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. II. The significance of trophically available metal (TAM). Mar Ecol Prog Ser 257:125–135

Wallace WG, Lee BG, Luoma SN (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar Ecol Prog Ser 249:183–197

Wang W-X (2013) Prediction of metal toxicity in aquatic organisms. Chin Sci Bull 58:194–202

Wang W-X, Rainbow PS (2008) Comparative approaches to understand metal bioaccumulation in aquatic animals. Comp Biochem Physiol 148:315–323

Wei Y, Zhang J, Zhang D, Tu T, Luo L (2014) Metal concentrations in various fish organs of different fish species from Poyang Lake, China. Ecotoxicol Environ Saf 104:182–188

Weiner ER (2000) Applications of environmental aquatic chemistry: a practical guide. Taylor & Francis Group, Boca Raton

Weltens R, Goossens R, Van Puymbroeck S (2000) Ecotoxicity of contaminated suspended solids for filter feeders (Daphnia magna). Arch Environ Contam Toxicol 39:315–323

Wilde KL, Stauber JL, Markich SJ, Franklin NM, Brown PL (2006) The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (Chlorella sp.). Arch Environ Contam Toxicol 51:174–185

Winner RW, Boesel MW, Farrell MP (1980) Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can J Fish Aquat Sci 37:647–655

Yamazaki M, Tanizaki Y, Shimokawa T (1996) Silver and other trace elements in a freshwater fish, *Carasius auratus langsdorfii*, from the Asakawa River in Tokyo, Japan. Environ Pollut 94:83–90

Zaccaro MC, Salazar C, Zulpa de Caire G, Storni de Cano M, Stella AM (2001) Lead toxicity in cyanobacterial porphyrin metabolism. Environ Toxicol 16:61–67

Zhang L, Qiu L, Wu H, Liu X, You L, Pei D, Zhao J (2012) Expression profiles of seven glutathione S-transferase (GST) genes from *Venerupis philippinarum* exposed to heavy metals and benzo [a] pyrene. Comp Biochem Physiol 155:517–527

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606:135–150