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Modelling in Metal Risk Assessment

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Abstract

Unique properties of metals that are largely different from the characteristics of organic substances should be considered in risk assessment. The bioavailability and toxicity of metals depend on their chemical speciation, that is, physical-chemical forms, in the environment, which is largely influenced by the environmental chemistry. Since metals in the environment are not always available while organisms have developed different processes to actively regulate the body burden, assessment of metal bioaccumulation might provide a better understanding of potential risks. Metal bioaccumulation is a prerequisite for metal toxicity, but is not the only determinant of metal toxicity. In addition to metal accumulation, metal toxicity is influenced by the subcellular partitioning of metals, which is controlled by the capacity of organisms to sequester and to detoxify metals. Different modelling approaches have been developed to address some of these factors. Both empirical and mechanistic equilibrium models have been developed and applied for characterising metal speciation in the environment. Metal bioaccumulation has been predicted by biodynamic models. The ability of organisms to detoxify metals has been taken into account in assessment based on the induction of metallothionein (MT) or subcellular partitioning. In addition, the interactions between organisms and metal ions have been taken into consideration in assessment of metal toxicity based on the accumulation of metal ions at biological surfaces.

Keywords: Metal, modelling, risk assessment, speciation, bioaccumulation, subcellular partitioning, toxicity

1. Introduction

Environmental pollution with metals is a serious problem in many areas in the world, and assessment of metal bioaccumulation and toxicity is of high concern. In such assessment, unique properties of metals that are largely different from the characteristics of organic substances

should be considered. The bioavailability and toxicity of metals are controlled by their chemical speciation, which is heavily influenced by environmental chemistry [1–5]. Metals are an intrinsic component of the environment, naturally occurring with varying background concentrations [6, 7]. Several metals are essential elements, that is, the elements that are present in living organisms and able to interact with the living system, and an insufficient amount of these elements leads to preventable or reversible effects on certain biological function, which do not occur at a physiological amount of the elements [8]. There is a limit to the regulation by the homeostasis mechanisms. Biological functions of organisms are affected by either the deficiency (below the limit) or toxicity (above the limit) of the essential metals. Because of these unique properties, organisms have developed various regulatory mechanisms for uptake, metabolism, distribution, storage, and elimination in response to metal exposure and to maintain metal homeostasis. The responses of organisms depend on a number of factors, from environmental conditions, bioaccumulation, to internal detoxification [9–11]. Metal bioaccumulation, a prerequisite for metal toxicity, is determined by the chemical speciation of metals in the environment, which depends on environmental conditions. In addition to metal accumulation, metal toxicity is influenced by metal subcellular partitioning. Different modelling approaches have been developed to address some of these factors as described in more detail in the following sections.

2. Metal availability in the environment

The bioavailability and toxicity of metals depend to a large extent on the chemical speciation of metals [12–15]. A widely recognised definition of the chemical speciation is that the chemical speciation of an element is the identification and quantification of the different, defined species, forms, or phases in which the element is present [16, 17]. Each form of the element is defined by its isotopic composition, electronic or oxidation state, or molecular structure [18, 19]. The mobility of metals in soil depends on the partition of metals between the solid and solution phases [20], which is determined by properties of the metals as well as composition of both solid and solution phases [21]. The most important soil properties include the total metal content, pH, cation exchange capacity (CEC), contents of soil organic matter (SOM), clay, oxides, and exchangeable Ca and Mg [22–26]. Besides the chemical extraction for direct determination, the solid-solution partitioning of metals in soils can be characterised by equilibrium models, which allow for predicting the speciation of a metal based on soil properties. Generally, two main approaches have been applied for modelling the solid-solution partitioning of metals in soils. They are based on: (1) empirical relations or transfer functions between the concentration of metals in soil solution or in a specific form and soil and solution properties [22, 24, 26, 27] or (2) complex process-based (mechanistic) multi-surface complexation models incorporating a detailed simulation of soil surface complexation and solution chemistry [23, 26, 28–31].

2.1. Transfer functions

The partition of metals between the solid and solution phases in soil has been expressed by adsorption isotherms or a distribution coefficient (K_d ; L/kg):

$$k_d = \frac{C}{C_{ss}} \quad (1)$$

where K_d (L/kg) is the distribution coefficient; C (mg/kg or mol/kg) is the total or reactive concentration of the metal in soil; C_{ss} (mg/L or mol/L) is the solution concentration.

In aerobic conditions, metals occluded in mineral particles and organic matter that are not readily available are included in the total pool, whilst the reactive fraction (extracted with 0.43M HNO_3) represents the metals available for exchange with soil solution with a particular time span [32]. Therefore, the reactive concentration has been used to substitute the total concentration in the above equation [32, 33]. The partitioning of metals in soil is usually expressed by sorption isotherms as described by a Freundlich equation:

$$C = k \cdot C_{ss}^n \quad (2)$$

where k and n are the Freundlich parameters. The exponent n reflects the variation in the binding strength with varying total pools. Equation 2 can be rewritten and extended to take into account the influence of soil properties [22, 32, 34–36]:

$$\log C = \log k + n \cdot \log C_{ss} + \sum a_i \cdot \log(X_i) \quad (3)$$

where a_i is the coefficient that reflects the influence of soil parameter X_i on the partitioning of metals between the solid and solution phases. The distribution coefficient has also been related to soil properties:

$$\log K_d = a_0 + a \cdot \log C_{ss} + \sum a_i \cdot \log(X_i) \quad (4)$$

Empirical relationships have been established between the distribution coefficient or the dissolved concentration, on the one hand, and the total/reactive concentration and soil properties, on the other. Different equations have been derived, depending on the empirical data that they have been based on, whilst a full description of soil properties is usually not available. Among soil properties, pH is the most commonly integrated parameter in transfer functions, followed by soil organic carbon (SOC), while other soil properties are rarely included in. This reflects the importance of pH and organic matter in determining metal speciation as well as the partitioning of metals between the solid and solution phases.

2.2. Mechanistic multi-surface complexation models

Substantial progress has been obtained in simulating the sorption of metal ions to soil and dissolved organic matter (DOM) with the development of different models, for example, MINEQL [37], GEOCHEM [38], NICADonnan [39], and WHAM V and VI [40, 41]. Metal speciation in soil has been characterised by various models calculating metal movement and available fractions and simulating impacts of physicochemical properties on metal dynamics in soil. The movement of different metal species in an environment is characterised by fate and transport models, while the partitioning of metals into dissolved, colloidal, and particulate phases is predicted by speciation/complexation models. Assemblage models have been formed by incorporating various surface complexation models. These process-based models provide a detailed description of metal complexation on different sorption surfaces [39, 42]. Surface complexation models based on thermodynamic processes have been developed for describing reactions of metals on the surface of soil reactive phases, such as SOM [39, 41], oxides [42, 43], and clay minerals [44]. Multi-surface complexation models have been formed from individual surface complexation models, assuming that the adsorption properties of soil can be characterised by the sum of the individual adsorption properties of individual components. Metal concentrations in soil solution have been predicted by using mechanistic geochemical models accounting for interactions of metals with various reactive surfaces [23, 24, 26, 29, 30].

Originally developed to characterise metal speciation in oxic waters [40, 41], WHAM has been applied to simulate the solid-solution partitioning [23, 45]. This model consists of several sub-models: Humic Ion-Binding Model V and models of inorganic solution chemistry, adsorption-desorption reactions of fulvic acids, precipitation of oxides, and cation exchange reactions on clays. In the Humic Ion-Binding Model V, humic substances are represented by molecules containing proton-dissociating groups that can bind to metal ions [40]. The binding of metal ions to humic substances is described in terms of complexation at discrete sites while taking into account both electrostatic interactions and competition among ions. In the application to soil systems, a fixed charge mineral cation exchanger was included to describe the presence of clays [46].

Weng et al. [29, 47] have proposed another multi-surface or mechanistic model for characterizing metal speciation in which soil is considered a set of independent sorption surfaces, that is, organic matter, clay silicates, and iron (hydr)oxides. Metal binding to these surfaces is simulated using advanced adsorption, cation exchange models, and default binding parameters. Humic acid is considered an analogue for SOM. The site density of SOM is assumed to be equal to 31% of the density of humic acid [47], while metal binding to SOM is described by using the non-ideal competitive consistent Adsorption (NICA)-Donnan model [39]. Clays are represented by illite because of their importance in Dutch soils [48]. The average charge of illite is assumed to be pH dependent (0.25 mol/kg; [49]). An electrostatic Donnan model is used to describe metal binding to clay. For amorphous iron (hydr)oxides, their amount is predicted as the oxalate-extractable Fe, and their surface area is considered similar to that of hydrous ferric oxides (HFO; 600 m²/g) [42]. For crystalline iron (hydr)oxides, their surface area is assumed to be the same as that of goethite (100 m²/g), and their amount is calculated as the difference between total (aqua regia) and oxalate-extractable Fe. Metal binding to the oxides is simulated

using the two-site surface complexation diffuse double layer model [42]. The complexation of metals with carbonate and dissolved organic matter (DOM) is also taken into account in the model. DOM is assumed to consist of 30% humic acid and 30% fulvic acid [29].

The ORCHESTRA (Objective Representing CHEmical Speciation and TRANsport) modelling framework [50, 51] is a combination of different sub-models. Thermodynamic data from the MINTEQA2 [52] were used to calculate solution speciation. The sorption of ions to SOM and DOM was simulated with the NICA-Donnan model [39, 53, 54] using generic binding parameters and constants derived by Milne et al. [55, 56]. When data on DOM were not available, DOM was assumed to consist of 50% reactive humic substances. In addition, SOM and DOM were represented by humic acid assuming that humic acid consists of 50% carbon [56]. The sorption of ions on the surface of (hydr)oxides was described by the generalized two layer model (GTLM) of Dzombak and Morel [42]. Site densities of the amorphous iron and aluminium (hydr)oxides were calculated from the assumed specific surface area of 600 m²/g for hydrous ferric oxide [42]. Crystalline iron (hydr)oxides were represented by the hydrous ferric oxide, assuming the same reactivity. Moreover, a specific surface area of 100 m²/g was assumed for these (hydr)oxides [43]. The nonspecific sorption of ions to permanently charged clay surfaces was modelled with a Donnan model. The illitic clay mineral with an average charge density of 0.25 eq/kg and a fixed Donnan model of 1 L/kg were used to represent the clay mineral [49].

3. Metal bioaccumulation

Since metals in the environment are not always available, assessing metal accumulation in sentinel species is one of the most effective methods for evaluating effects of metals on biota. The tendency of chemicals to be accumulated in organisms is usually expressed by the bioaccumulation factor (BAF) and the bioconcentration factor (BCF). These factors represent the ratio of the residue in the organisms versus the concentration in water at equilibrium, including or excluding the uptake from dietary sources, respectively. For metals, BCF and BAF vary widely and are inversely related to the external water concentration [57–59]. These characteristics of the accumulation factors for metals are attributed to the complex mechanisms of metal uptake and accumulation [60]. In a number of studies, uptake via saturable kinetics-exhibiting mechanisms has been shown to be more common and toxicologically relevant than passive diffusion [61–65]. The concentration of metals accumulated in organisms is further influenced by different physiological and anatomical mechanisms in response to metal exposure [6]. For essential metals, organisms are able to actively regulate metal bioaccumulation and maintain homeostasis over a range of exposure via exclusion or increased elimination [11, 57, 65]. Organisms have also developed different mechanisms to sequester, detoxify, or store excess metals [11, 62, 66]. The dependence of BCF and BAF on exposure conditions as well their inverse relationship with the exposure concentration invalidates the use of these factors in risk assessment [6, 57, 59].

Biodynamic models have been recommended as an alternative to single and generic values of BCF or BAF in the assessment of metal bioaccumulation [67]. Such models allow for integrating

and distinguishing different exposure routes (e.g., water vs. diet) and the dynamic nature of bioaccumulation processes [6, 68, 69]. The models provide a better understanding of the extent of and the contributors to the variability in the bioaccumulation among metals in various species and at different conditions [67, 70]. The biodynamic models are based on the concept of biodynamics that the accumulation of chemicals occurs as a result of a balance of different fluxes. The models assume that the rate of the fluxes can be realistically determined in controlled experiments with varying concentrations and conditions [67]. Uncertainties are inherent in the application of these empirical rates to different conditions, especially those that are extremely different from the conditions in which the rates have been derived. This limitation can be eliminated by integrating inverse phenomena into unifying concepts. The biokinetic model based on a unifying concept is considered 'mechanistic' from the perspective that physiological rates are estimated from chemical-specific properties of substances and species-specific physiological characteristics of organisms [71, 72]. This method facilitates extrapolation to a wide range of chemicals, organisms, and environmental conditions, without the necessity for case-specific calibration. Quantitative relations between uptake and elimination rate constants, on the one hand, and metal-specific properties and species-specific physiological characteristics, on the other hand, are required for the development of a mechanistic model. Some potential for meeting this requirement has been revealed from the findings during the last decades. Specifically, metal absorption and elimination rates have been related to the filtration rate and species weight, respectively [71, 73]. The affinity of metals for proteins, which are intensively involved in trafficking processes of metals, may explain the uptake rate constant from the dissolved phase [72]. The affinity of metals for the proteins depends on metal charge and atomic radius and reflects the preferences for coordination and ligands [72, 74, 75]. The suggestion on a potential correlation between uptake kinetics and the affinity of metals for protein has been substantiated by the reported relationship between the absorption efficiency and the binding of metals to membrane transport proteins [76]. Efforts have been put in developing bioaccumulation and toxicity models based on the affinity of metals for biological ligands [69, 72, 77]. Different indicators of metal binding to biological ligands and bioaccumulation have been related to various chemical properties of metals [78–82].

The accumulation of metals in organisms occurs as a result of a balance of the uptake from food as well as water and losses. Moreover, metal concentrations in organisms are affected by the growth dilution. Taking these factors into consideration, the concentration of metals accumulated in organisms (C ; $\mu\text{g/g dw}$) can be expressed by the following equation:

$$\frac{dC}{dt} = (k_u \times C_w) + (IR \times AE \times C_f) - (k_{ew} + k_{ef} + g) \times C \quad (5)$$

where k_u (L/g dw/d) is the absorption rate; C_w ($\mu\text{g/L}$) is the dissolved metal concentration; IR (g/g dw/d) is the ingestion rate; AE (/) is the assimilation efficiency; C_f ($\mu\text{g/g}$) is the metal concentration in food; k_{ew} (1/d) is the excretion rate; k_{ef} (1/d) is the egestion rate; and g (1/d) is

the growth rate. The instant concentration of metals in organisms can be solved from Equation 5:

$$C = \frac{(k_u \times C_w) + (IR \times AE \times C_f)}{k_{ew} + k_{ef} + g} \times \left(1 - e^{-(k_{ew} + k_{ef} + g) \times t}\right) \quad (6)$$

The first factor in Equation 5 represents the uptake from the dissolved phase and can be further elaborated based on the absorption efficiency and the filtration rate [69]. In the study of Le et al. [69], the filtration, ingestion, and growth rates were related to the species weight while the absorption and assimilation efficiency was considered metal specific. In addition, elimination rates were parameterised based on both chemical properties of metals and mussel size.

Such a mechanistic model has shown good potential for predicting metal accumulation in the zebra mussel with different size and from various sites. A mechanistic model, which is based on chemical properties of metals and physiological characteristics of organisms while taking site-specific contamination levels into consideration, facilitates a wide extrapolation to different conditions and metals. However, a number of disadvantages are inherent in the current model. The modelling is based on the dissolved metal concentrations without a specification of chemical species and forms of metals in the environment. Although different uptake pathways are included in the model, a distinction of tissue-specific accumulation of metals is not accounted for. Another disadvantage of the model is related to the derivation of the relationship between the rate of physiological processes and the chemical properties of metals. Despite the potential correlation between the uptake kinetics and some chemical properties of metals shown recently, quantitative relationships established between these two factors have hardly been validated. Moreover, relationships between uptake kinetics and chemical properties of metals have usually been derived based on limited experimental data, thereby leading to intrinsic uncertainties in the application of the derived relationships.

4. Metal subcellular fates and partitioning

4.1. Subcellular partitioning

Bioaccumulation is a prerequisite, but not necessarily a reliable indicator of metal toxicity due to the species-specific capacity of organisms to detoxify the metals accumulated [9, 59, 82]. The detoxifying mechanisms have been suggested to account for the tolerance of organisms [83]. Besides metal uptake, metal toxicity is determined by the subcellular partitioning of metals in organisms [83]. Metals accumulated are distributed to different cellular components and sequestered by binding to proteins or peptides (e.g., metallothionein and glutathione) and granules [10, 83–86]. Therefore, only parts of metals are accumulated in sensitive cellular fractions [9]. From a toxicology perspective, metals accumulated have been divided into two fractions, that is, metal-sensitive fraction (MSF) and biologically detoxified metal (BDM) [83, 87, 88]. The former consists of metals in mitochondria, associated with heat-denaturable

proteins (HDPs), lysosomes, and microsomes. The latter includes metals in association with heat-stable proteins (HSPs) or metallothionein (MT)-like proteins and metal-rich granules.

It has been suggested that sub-lethal toxicity is accompanied with changes in subcellular partitioning, especially when the threshold is exceeded, that is, saturation of detoxification mechanisms [83]. Eyckmans et al. [89] showed the relationship between the tolerance of three freshwater fish species to excess Cu and the subcellular partitioning of Cu. The subcellular partitioning of metals may divulge potential mechanisms of toxicity as well as the fate of accumulated metals [90]. For instance, the binding of metals to cytosolic proteins such as MT has widely been demonstrated to alleviate toxic effects [91]. In contrast, the association of metals such as Cd to mitochondria modulates oxidative phosphorylation, followed by decreases in ATP production [92]. Similarly, the binding of Cd to the nucleus is potentially toxic because it can lead to DNA damage and stimulate mutagenesis [93, 94]. Different ligands are included in the HSP fraction, that is, amino acids, glutathione, and metallothioneins [95]. This fraction determines the tolerance and resistance of organisms to metals [83, 84, 87]. The relationships between subcellular partitioning and metal toxicity are complicated because of the different physiological functions of each fraction. The accumulation of metals in the lysosomes and microsomes might reflect the storage for eventual elimination if metals are mainly in the lysosomal fraction [85, 96]. In contrast, metals associated with microsomes can indicate toxicity because of the presence of fragmented endoplasmic reticulum, which is involved in the synthesis and transport of proteins [85, 87]. In addition, mitochondria is the most sensitive fraction [85]. Because of these factors, subcellular partitioning has recently been included in the assessment of metal toxicity.

4.2. Assessment based on metallothionein induction

The induction of MT has been integrated in various monitoring programmes and ecotoxicological assessment. However, recent reviews on the use of MT induction as biomarkers of metal exposure and toxicity raise questions on the validity of this method [11, 97]. The main concern comes from the lack of well-described time- and dose-dependent MT induction, while the induction of MT widely varies, depending on metals, species, and environmental conditions [97]. Further concern results from the lack of well-described relationships between MT induction and metal exposure, metal accumulation, and biological effects [11]. The validity of the use of MT induction as a biomarker is questionable because of the lack of enhancement in MT induction in response to metal exposure and/or the insignificant relationships between metal and MT concentrations [11]. As explained in the reviews by these authors, these results are attributed to different factors determining the cytosolic free metal ion concentrations, the presence of different MT isoforms in different tissues with various physiological functions, and turnover kinetics of MT as well as the high variability of MT induction and the basal level [11]. These factors should be taken into consideration in the assessment of metal exposure and toxicity based on MT induction. Another concern is related to the selection of organs/tissues as the target in the assessment. The digestive gland has widely been used as the target tissue [98, 99] from the point of view that this is the long-term storage organ. In addition, this organ plays an important role in different processes such as immune defence, homeostasis, xenobiotic

mechanisms, elimination, and detoxification [100–103]. Another reason for the use of the digestive gland is that this organ has the highest amount of proteins, including MT [104]. However, the suitability of the digestive ligand is doubtful because of the influence of natural factors and physiological changes on the induction of MT in this organ [104, 105]. As a major tissue for metal uptake, the induction of MT in gills is expected to represent the response of organisms to the ongoing exposure or accidental pollution [106]. Because of these issues, a single value of MT concentrations in the whole soft tissue or in a specific tissue is not always good indicator of metal exposure or effects on biota. As suggested by Le et al. [11], the induction of MT needs to be considered in relation to a number of factors as briefly mentioned above.

4.3. Assessment based on subcellular partitioning

Binding to MT is not the only mechanism for organisms to detoxify and to sequester excess metals. Metals can be associated with insoluble complexes in granules or lysosomes as mentioned above. The proportion of metals bound to MT is not always the dominant fraction and so MT induction is not necessarily a reliable indicator of metal exposure and biological effects. According to Adams et al. [107], the kinetic partitioning of metals into MSF and BDM is the key factor for an understanding of the exposure-toxicity relationship. This has been demonstrated by the shift of Cu accumulated in gills of common carp from MSF to BDM in the first period of the Cu exposure [89]. The ‘Spillover’ hypothesis has been used to simulate the relationship between metal bioaccumulation and toxicity. This term indicates the situation when the capacity of organisms to detoxify excess metals is overwhelmed, thereby resulting in adverse effects [66]. From a broad perspective, spillover is assumed to occur when the uptake rate exceeds a combination of the detoxification and elimination rates, leading to the accumulation of metals in the MSF fractions [9, 108]. This hypothesis has been used in some approaches for assessing metal toxicity.

The first approach is based on the ratio between MSF and MDP fractions or the relative contribution of the MSF and MDF fractions in other words [89, 90]. This approach is simplistic and does not provide a full description of toxicity [90]. There is not constant partitioning between MSF and BDM. Metals can be exchanged between the two fractions, and only a small fraction of metals occurs as free ions [90]. The use of the relative distribution between these two pools may lead to misinterpretation of spillover [90, 92, 109]. For instance, the increase in the amount of metals bound to MSF might be hidden by the lack of changes in the relative proportion, while the accumulation in all compartments increases. This has been illustrated by increases in metal concentrations in both BDM and MSF fractions in yellow eels [110], mummichogs [111], or yellow perch [85].

The second approach is to develop a relationship between MSF and toxic effects [112], assuming that adverse impacts on organisms increase with increasing amounts of metals in MSF. This method is based on the hypothesis that spillover occurs when certain detoxification mechanisms are overwhelmed and excess metals are accumulated in MSF, leading to adverse effects.

The third approach is based on the kinetics of metal accumulation in MSF [108, 113, 114]. The accumulation of metals in MSF can be described as a balance of uptake (e.g., from the dis-

solved), losses via elimination, and the partitioning of the metals to BDM, expressed by the detoxification rate:

$$MIT = (k_u \times C_w) = (k_{detox} + k_e) \times C_{IT} \quad (7)$$

where MIT (mol/g/d) is the metal influx threshold ; k_u (L/g/d) is the uptake rate constant; C_w (mol/L) is the dissolved metal concentration; k_{detox} (1/d) is the detoxification rate constant; k_e (1/d) is the elimination rate constant; C_{IT} (mol/g) is the metal accumulation in MSF at the influx threshold.

This approach is supported by a negative correlation between elimination and detoxification [113, 115]. However, disadvantages are inherent in the assumption of this method that spillover occurs when the uptake rate exceeds combination of the elimination and detoxification rates, that is, no metal is accumulated in MSF below the threshold. Previous studies have indicated that spillover does not happen at low-exposure concentrations. In other words, there is no threshold below which the accumulation of metals in the sensitive fractions does not occur [84, 85].

5. Biological responses to metal exposure

Available approaches based on the induction of MT or taking into account subcellular partitioning of metals have shown some limitations in predicting metal toxicity as mentioned in the previous section. Moreover, the target organ or tissue is species specific, depending on the kinetics of metal accumulation, that is, the differences in absorption, distribution, and excretion [6]. A method that has been demonstrated to be more applicable to different species is to predict metal toxicity based on the accumulation of metals at biological surfaces, which allows for taking into account interactions between organisms and metals at biological surfaces.

5.1. Biotic Ligand Model

Interactions at the water-organism interface have been integrated in the Biotic Ligand Model (BLM). The conceptual framework of the BLM originates from two models: the gill surface interaction model and the free ion activity model (FIAM) [116–118]. The FIAM model assumes that free ions are the main reactive species of metals, determining metal bioavailability and toxicity [116, 117]. The FIAM model has then been extended to take into account the interactions of metals with organisms. One example is the fish gill surface interaction model [119], which has been developed by integrating conditional metal-gill surface binding constants to a geochemical speciation model. On the basis of the fish gill surface interaction model, the BLM has been developed to facilitate the application to various species, metals, and exposure conditions as well. According to the concept of the BLM, environmental geochemistry as well as toxicology principles are taken into consideration in determining the fraction of metals that

provokes effects on biota [119–121]. Initial toxicology bases of the model were effects of trace metals on ionoregulation. Apical (e.g., Na⁺ and Ca²⁺ channels) and basolateral (e.g., Na⁺/K⁺- and Ca²⁺-ATPase) transport channels, exchangers, and enzymes are negatively charged proteins that potentially bind to metal cations [122].

With the principles mentioned above, the BLM has shown substantial advantages in assessment of metal bioavailability. By including interactions of metal ions with both biotic and abiotic ligands, the BLM might provide more reliable estimates of metal bioavailability and toxicity. Moreover, the accumulation of metal ions at the physiological active sites of toxic actions, which determines toxic effect, is, in principle, distinguished from the total body burden [6]. Another advantage of the BLM is the inclusion of environmental conditions in estimating metal bioavailability and subsequent application to site-specific assessment [6]. Furthermore, the BLM provides a mechanistic understanding of metal-organism interactions as well as a mechanistic interpretation of metal toxicity, as illustrated by the results in the study of Le et al. [123]. The BLM has potential for assessing the toxicity of metal mixtures [123, 124]. Norwood et al. [124] suggested that metal-metal interactions can be predicted based on known stability constants. If two metals compete for the same binding site, the total amount of the metals bound to the biotic ligands determines combined effects [123]. Alternatively, the accumulation of individual metals at the biotic ligands can be used as inputs to the response addition model for estimating toxicity of metal mixtures [124, 125]. This suggestion has been demonstrated by the increasing application of the BLM for predicting joint toxicity of metals. Liu et al. [126] predicted the toxicity of metal mixtures based on the simple sum of the fraction of biotic ligands occupied by individual metals. This approach allows for taking into account the metal-specific affinity for binding sites of biotic ligands, but not the metal-specific toxic potency. In other studies, the BLM concepts are usually combined with conventional concepts of mixture toxicity such as concentration addition. As such, the specific toxic potency of metals is taken into account in the estimates. For instance, the toxicity of metal mixtures has been related to the toxic equivalent quotient (TEQ), which is based on the accumulation of metal ions at the binding sites of biotic ligands while giving consideration to the metal-specific toxic potency [123, 126]. The toxic unit (TU) is the concept mostly integrated in the BLM-based approaches for estimating joint toxicity of metals.

5.2. The electrostatic model

The electrostatic model originates from the reported differences between the ion concentration at the root plasma membrane surface and the ion concentration in the external medium, which is induced by the negative charge at the plasma membrane surface [127, 128]. The surface potential affects the activity of ions at the plasma membrane surface via electrostatic attraction or repulsion. In addition, the potential influences the difference in the electrical potential across the membrane, which stimulates the transport of ions through the membrane. A number of studies have shown the effects of electrical potential at the plasma membrane surface on cation uptake [127, 128]. The principle effect of the electrical potential at the plasma membrane surface is to control the ion activity at the surface [129].

The model allows for integrating plant-ion interactions in predicting metal toxicity [130–132]. In addition, the model gives consideration to the interactions among ions while estimating metal toxicity [132]. The electrostatic model has been applied to simulate effects of major cations on the toxicity of trace metal ions [131–134]. According to the principle of the electrostatic model, additions of cations to the bulk medium reduce the negativity of the electrical potential at the plasma membrane surface, thereby leading to decreases in the negativity of the electrical potential at the plasma membrane surface accompanied by reduced accumulation of trace metal ions at the plasma membrane surface and subsequent alleviation of toxic effects [135]. The electrostatic approach therefore might provide additional explanation for interpreting ion-ion interactions as well as effects of major cations on the toxicity of trace metal ions besides the competitive binding assumed in the BLM. According to the electrostatic principles, three mechanisms have been suggested to account for ameliorative effects of Ca^{2+} on metal toxicity [128]. The first mechanism is the electrostatic displacement of trace metal ions at the plasma membrane surface by Ca^{2+} . The second is the restoration of Ca^{2+} at the cell surface in response to low levels of surface Ca^{2+} . This mechanism is mediated in order to avoid the inhibition of the plant growth induced by the low contents of Ca^{2+} at the cell surface. The third mechanism covers other types of interactions between Ca^{2+} and trace metal ions, such as the blockade of the ion channel [136]. Despite the toxicant- and major cation-specific relative importance of these mechanisms, mechanism 1 generally occurs in all cases while the contribution of mechanism 2 is minor [128]. The significance of mechanism 3 is trace metal ion specific. The relative influence of major cations such as Ca^{2+} and Mg^{2+} on the toxicity of trace metal ions varies, depending on the trace metal ions and species [128]. For instance, Ca^{2+} has stronger effects on the toxicity of Al^{3+} to soybean than Mg^{2+} [137, 138]. In wheat, Mg^{2+} is more effective than Ca^{2+} in inhibiting Zn^{2+} toxicity, whereas Mg^{2+} does not have effects on the toxicity of Al^{3+} [128].

5.3. WHAM-based bioavailability model

Originally developed for determining and quantifying chemical forms and species of metals in different phases in the environment, speciation models such as WHAM have recently been used for estimating metal bioavailability and toxicity (hereafter referred to as the WHAM-based model). In this method, humic acid is considered a surrogate to biological surfaces [139–144]. As such, the amount of metals bound to humic acid computed by WHAM is used for estimating metal toxicity for different species. In the application of the WHAM-based model to plants, humic acid is used as a surrogate for the root surface [144]. In other words, the interactions of metal ions and the root surface are simulated by the interactions with humic acid.

The WHAM-based model was initiated from the similarities in terms of chemical structure between the root surface and humic acid. The root surface is a heterogeneous mixture of various metal-binding functional groups [145–147]. Similarly, humic acid possesses a heterogeneous mixture of functional groups, mainly carboxylic and phenolic acids [141]. Furthermore, the ratio of 1:2 between the site densities of phenolic and carboxylic groups assumed in WHAM [40, 41] lies in the range from 0.49:1 to 1:1 reported for root cell walls of different plant

species [148]. Because of this similar structure of the root surface and humic acid, metal accumulation on the root surface and metal binding to humic acids are influenced by similar factors. These factors include electrostatic interactions and chemical heterogeneity [127, 128, 149, 150]. The relevance of using metal binding to humic acid to represent metal accumulation at biological surfaces is further substantiated by the nature of the sorption of metal cations to the biological surfaces [151].

The WHAM-based model allows for the interactions between metal ions to be integrated in modelling metal toxicity [144]. Specifically, interactions between ions are considered in modelling metal binding to humic acid in WHAM and therefore accounted for in estimating metal accumulation at the biological surfaces. Another advantage of the WHAM-based model is related to the availability of binding constants in WHAM, which facilitates a wide application to different metals [144]. Moreover, previous studies have shown small variations in the binding constants among different species [145, 147, 152, 153]. For instance, the logarithm of the binding constants of Cd to the carboxylic group on the membrane surface of bacteria, fungi, and plants were in the range 3.3–3.5. Moreover, the cell walls of algae, maize, soybeans, and higher plants have similar titration curves [154–156]. These results indicate the applicability of a single set of binding constants to different organisms.

In the study of Le et al. [144], the amount of metals bound to humic acid was computed with WHAM in which metal sorption to humic substances is simulated by using a structured formulation of discrete, chemically plausible binding sites for protons and metals. This allows the creation of regular arrays of bidentate and tridentate binding sites for metals. Metal aquo ions compete with their first hydrolysis products, protons, and other metals for binding sites. In addition to the intrinsic metal binding strength to uncharged molecules, electrostatic effects were taken into consideration while predicting metal binding to humic acid. Le et al. [144] showed the potential of the WHAM-HA model for predicting both total and internalised metal concentrations in roots. Another example of the approaches based on the geochemical equilibrium in WHAM is the WHAM- F_{TOX} model for estimating metal toxicity [140, 143]. In this approach, mixture toxicity was simulated as a function of the metal-specific toxic potency and the amount of metal ions bound to the biological surfaces computed with WHAM.

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