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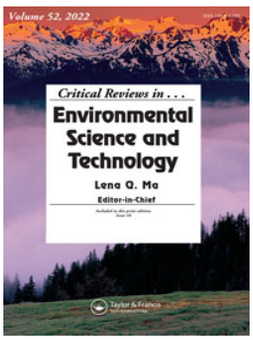

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Incorporation of chemical and toxicological availability into metal mixture toxicity modeling: State of the art and future perspectives

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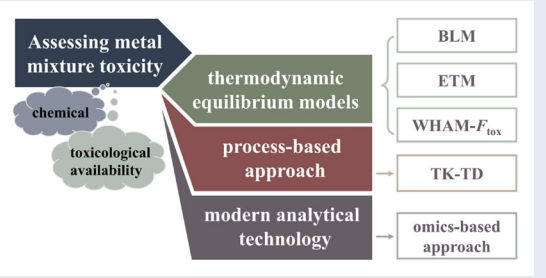
ABSTRACT



In the real world, metals are generally present as mixtures, but evaluating their mixture toxicity is still a daunting challenge. The classic conceptual models of concentration addition (CA) and independent action (IA) have been widely used by simply adding doses and responses to predict mixture effects assuming there is non-interaction. In cases where interactions do occur in a mixture, both CA and IA are no longer applicable for quantifying the toxicity, because interpretation of the observed joint effects is often limited to overall antagonism or synergism. In metal mixtures, interactive effects may occur at various levels, such as the exposure level, the uptake level, and the target level. A comprehensive understanding of the mechanisms of joint toxicity is therefore needed to incorporate the interactive effects of mixture components in predicting mixture toxicity. With this in mind, numerous bioavailability-based methods may be considered, with diverse mechanistic perspectives, such as the biotic ligand model (BLM), the electrostatic toxicity model (ETM), the WHAM-F_{tox} approach, a toxicokinetic-toxicodynamic (TK-TD) and an omics-based approach. This review therefore timely summarizes the representative predictive tools and their underlying mechanisms and highlights the importance of integrating mixture interactions and bioavailability in assessing the toxicity and risks of metal mixtures.

KEYWORDS Biotic ligand model; electrostatic; mixture effects; omics; toxicokinetic-toxicodynamic; WHAM

1. Introduction

In the environment, metals are seldom present in isolation, therefore exposure to multiple metals is a rule rather than an exception (Kortenkamp et al.,

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2009). Given the potential of metals to pose detrimental impacts on humans and the environment, evaluating their effects as mixtures is urgently needed (Escher et al., 2020). However, the existing chemical regulations mainly focus on single metals and rarely consider mixture exposure scenarios, which may have little environmental relevance. It is impractical to evaluate all possible mixtures, because there are innumerable combinations based on the fluctuating concentrations of single metals in mixtures (Chen et al., 2013). Thus, there is an urgent need for developing simple and efficient models to decipher and predict the mixed effects of metals (Baas et al., 2009; Farley et al., 2015; Meyer et al., 2015).

The conceptual models of concentration addition (CA) and independent action (IA) have been most widely applied to predict the effects of metal mixtures. The CA concept was developed by Loewe and Muischnek (1926) to describe mixtures of components having the same or a similar mode of toxic action (i.e., acting on the same biological pathway and strictly on the same molecular target). CA assumes that the relative toxicity of the metals that are present in mixtures is the same as their relative toxicity when present individually. The concept of IA was first proposed by Bliss (1939) to describe mixtures of components having different modes of action (i.e., acting on different physiological systems). IA addresses the question whether the probability of being affected by one metal may be independent from the probability of being affected by another metal. In this model, the relative toxicity potency of metals is ignored, and the mixture effect is predicted from the joint probabilities of statistically independent events (Peijnenburg & Vijver, 2007). Both approaches are based on the assumption that components in the mixture do not physically, chemically, or biologically interact. With regard to the choice of a conceptual model, the basic idea is to use CA if the mixture components are expected to act similarly and to use IA if they are expected to act dissimilarly (Junghans et al., 2006). However, identifying the modes of action for different chemicals is not always possible. In those cases, CA is suggested to be the more conservative choice in a risk assessment context as it estimates higher toxic effects than IA and therefore represents the worst-case scenario for assessing mixture exposures (Backhaus & Faust, 2012; Cremazy et al., 2018; Gopalapillai & Hale, 2017; Jegede et al., 2020; Lock & Janssen, 2002; Nys et al., 2017).

To date, our understanding of mixture toxicity is still based on these concepts, with concentration addition as the basis for most models (Vijver et al., 2010). However, actually, the joint toxicity of metals may not correspond to effects predicted based on "additivity" without considering interactive effects (Cedergreen et al., 2017; Kamo et al., 2019; Traudt et al., 2017). The interactive effects of mixture components may lead to more-than-additive (synergism) or less-than-additive (antagonism) effects and

possibly occur at various levels (such as the exposure level, the uptake level, and the target level) (Weltje, 1998). Specifically, the exposure level describes physicochemical interactive effects in the exposure media, affecting chemical speciation and hence the bioavailability of metals. In natural environments, environmental (geochemical) processes logically affect this type of interactions, and this complicates the simplification of the interactions. Apart from this, multienvironmental factors, such as organic carbon content, alkalinity, and pH, can also influence metal speciation and bioavailability, thus altering the first type of interactions. The uptake level deals with physiological interactive effects during the uptake processes, influencing toxicokinetic processes and thereby the available amount of metal reaching the sites of action. The target level involves interactive effects of metals at the target sites within an organism, which affect toxicodynamic processes and subsequently the combined effect (Conder & Lanno, 2000; Kinraide, 1998). Insight into these interactive effects levels and their relative importance is of great value for the toxicity assessment of metal mixtures. This information will help to generalize study results on metal mixtures, as well as for different exposure conditions and organisms.

For an effective and accurate risk assessment of metal mixtures, appropriate models or tools are required that enable the prediction of mixture effects, which cover both simple and complex mixtures and incorporate mixture interactive effects. Many mechanistically underpinned models based on different perspectives have thus been developed to predict the mixture toxicity of metals considering the interactive effects of mixture components, including: a) thermodynamic equilibrium models (e.g., biotic ligand model (BLM) (Di Toro et al., 2001), electrostatic toxicity model (ETM) (Wang et al., 2008) and WHAM-F_{tox} approach (Stockdale et al., 2010)); b) process-based approaches (e.g., toxicokinetic-toxicodynamic (TK-TD) model (Jager et al., 2011)); and c) modern analytical technologies (e.g., omics-based approaches (Ankley et al., 2006)). From the thermodynamic equilibrium models, it is suitable to apply the BLM-based approaches to interpret mixture effects, postulating that competition is responsible for metal mixture interactive effects (Niyogi & Wood, 2004). The ETM assumes that metal toxicity and uptake are determined by the ion activity at the surface of the cell membrane. Cations (e.g., Ca^{2+}, Mg^{2+}, and H^{+}) in the bulk solution can reduce the negativity of the electrical potential at the surface of the cell membrane by charge screening and ionic binding (Kinraide, 1998; Wang, Kinraide et al., 2011), which, in turn, can reduce metal ion activities at the membrane surface. Therefore, the ETM modeling approach allows incorporating the effects of various cations simultaneously in modeling mixture toxicity and may provide mechanistic insights (in addition to competitive binding) into mixture interactive effects at the

boundary layer surrounding the cell surface. The WHAM-F_{tox} serves as an innovative bioavailability-based model (Stockdale et al., 2010). It is assumed that the interactive effects between metals and biological surfaces can be reflected by the interactive effects with particulate humic acid (HA) (Stockdale et al., 2014). HA contains various functional groups and can represent the heterogeneous distribution of biotic ligand sites. It should be noted that the mixture toxicity of metals to certain endpoints is predicted by these thermodynamic equilibrium models without considering the influence of time, which is of great significance for quantitative risk assessments (Di Toro et al., 2001; Slaveykova & Wilkinson, 2005).

In fact, metal bioavailability influenced by interactive effects is not a static but rather a dynamic phenomenon. For better understanding and predicting the mixture toxicity of metals, the underlying interactive effects during TK and TD processes deserve further investigation. Consequently, the process-based TK-TD model is proposed to estimate the real-time toxicity of metals by simulating the time-course processes (Ashauer & Escher, 2010). This approach enables the extrapolation of metal toxicity in the course of time and toward higher organisms. Considering process-based interactive effects, the development of predictive approaches is more powerful to unravel the underlying mechanisms of metal mixture toxicity coming at a price in time-consuming and costs in ecotoxicological testing.

Moreover, interactive effects of metals with biological target sites (e.g., protein, DNA, and ion channel) are the basic steps for inducing toxic effects. At this phase, interactive effects between metals and the target species affect the toxicity of metal mixtures. Interactive effects of metals at this level together with interactive effects of metal-metal may result in different patterns of mixture toxicity, e.g., additive, less-than-additive, and more-than-additive. Metals in a mixture may have many or uncertain modes of action when they interact at the receptor sites. To investigate these potential interactive effects, novel toxicogenomic approaches have been developed in recent years (Garcia-Sevillano et al., 2014). These approaches can help to provide a basis for deciphering the mechanisms involved in mixture toxicity. Genomics technologies (e.g., global gene expression) are applied to investigate adverse impacts of metals (Suter et al., 2004). This approach integrates conventional toxicology and promising technologies of genomics and bioinformatics. The association of metal mixture toxicity with mechanisms of interactive effects at the molecular level can be identified through detecting gene expression changes after exposure to the individual metals and their mixtures. Genomes, including overall hereditary information of organisms, are generally found in the DNA or RNA and include genes and non-coding order of the DNA or RNA, which contain information for building and maintaining organisms (Wu et al., 2016). Therefore,

environmental circumstances of the organisms together with their genes, proteins, and biochemical pathways are considered in the toxicogenomic approach. This method can effectively link environmental conditions to phenotypes by linking the structure of the genome to phenotypes based on genes, proteins, and biochemical pathways.

Given the global desire of minimizing animal testing and reducing costs of regulatory testing (Hofer et al., 2004), modeling approaches are favorable for conducting ecological risk assessments of metals. Because of the extensive relevant literature over the past few decades, it is impossible to be comprehensive in this review. Instead, this review will specifically focus on summarizing the representative predictive tools for assessing the toxicity of metal mixtures by considering mixture interactive effects and bioavailability. In addition, the underlying mechanisms as well as the recent and new applications of these models are also included, which will help to provide guiding principles for future research on the toxicity of a 'cocktail' of metals, representatives of real environmental exposure scenarios.

2. Thermodynamic equilibrium models

2.1. Biotic ligand model (BLM)

2.1.1. Basic concepts and principles

The BLM is initially a theoretical framework in which toxicity is related to the binding of metal ions to the sites of toxic action on an aquatic organism. It is often used as the state-of-the-art approach to quantify the link between metal toxicity and chemical availability in aquatic systems (Di Toro et al., 2001; Paquin et al. 2002). It is a synthesis of decades of work on metal speciation, bioaccumulation, toxicity and physiology (Paquin et al. 2002). The principal feature in the BLM is the competition of the free metal ion with other cations for binding at the biotic ligand (Figure 1). This feature distinguishes the BLM from earlier concepts that considered only the free metal ion as the toxic species. According to the assumption of the BLM, metal ions (M^{z+}) and other cations (H^+, K^+, Ca^{2+}, Na^+, and Mg^{2+}) can bind to the theoretical biotic ligand (BL) sites (Di Toro et al., 2001). The interactive effect between cations and BL is treated as a surface complexation reaction. At equilibrium, for example, the stability constant for the binding of M^{z+} to biotic ligands, K_{MBL} (L/mol), can be expressed as a function of the concentrations of cation-biotic ligand complexes [MBL] (mol/L) and unoccupied biotic ligand sites [BL] (mol/L):

$$K_{MBL} = \frac{[MBL]}{\{M^{z+}\} \times [BL]} \quad (1)$$

where $\{M^{z+}\}$ is the free metal ion activity (mol/L).

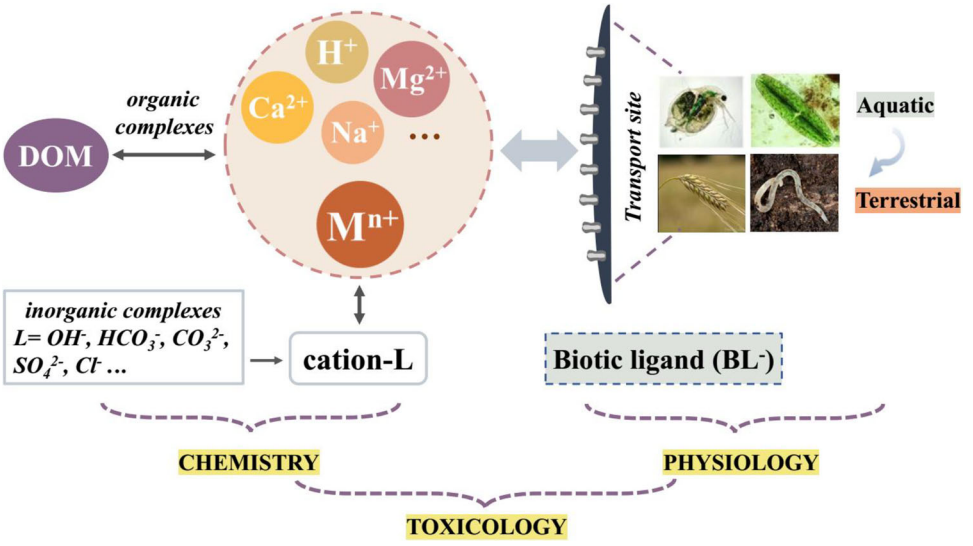

Figure 1. Brief description of the Biotic Ligand Model (BLM). DOM = Dissolved Organic Matter, L = ligand.

Metal toxicity is assumed to be proportional to the fraction (f) of the total number of biotic ligand sites $[BL]_T$ occupied by the metal. The f value depends on the binding affinity of M^{z+} to the BL and the presence and binding affinity of the competing cations (De Schamphelaere & Janssen, 2002):

$$f = \frac{[MBL]}{[BL]_T} = \frac{K_{MBL} \times \{M^{z+}\}}{1 + K_{MBL} \times \{M^{z+}\} + \sum K_{XBL} \times \{X^{z+}\}} \quad (2)$$

where $\{X^{z+}\}$ is the activity of major cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) in the solution, K_{XBL} are the binding constant of cations X^{z+} binding to the BL.

The value of f at the 50% effect level ($f_{MBL}^{50\%}$) is assumed to be constant according to the BLM theory. Eq. (2) then can be reorganized to:

$$EC50\{M^{z+}\} = \frac{f_{MBL}^{50\%}}{\left(1 - f_{MBL}^{50\%}\right) \times K_{MBL}} \left(1 + \sum K_{MBL} \times \{X^{z+}\}\right) \quad (3)$$

where $EC50\{M^{z+}\}$ is the free metal ion activity inducing 50% effect.

2.1.2. Application in assessment of metal toxicity

The principles underlying aquatic BLMs seem to be also valid for terrestrial species by regarding the active sites (i.e., biotic ligands) on or in organisms as more general binding sites. Several publications have been dedicated to a shift toward developing terrestrial BLMs for soil invertebrates (e.g., earthworm, enchytraeid, and collembola) (Li et al., 2008; Lock et al., 2006;

Steenbergen et al., 2005; Van Gestel & Koolhaas, 2004) and plants (Li et al., 2009; Lock et al., 2007; Thakali et al., 2006). It has been demonstrated to be theoretically and empirically feasible to extend them to terrestrial organisms. Given not enough soil toxicity datasets and much more complex research system than that for solution systems, it is not surprising that only few attempts have been done to apply the BLM concept to toxicity data in soil (An et al., 2012; Antunes et al., 2006; Plette et al., 1999). Unlike the aquatic system, not only the soil solution but also the soil particles can provide metals for terrestrial organisms (Steenbergen et al., 2005). The terrestrial BLM assumes equilibrium partitioning, akin to the aquatic BLM. This assumption may not be applicable if the complexation reactions between ions and biotic ligands at the surface of binding sites are slow relative to the internalization and the succeeding expression of the biological response. Moreover, the kinetics of metal dissolution from the solid phase to the solution phase can influence metal bioavailability in the soil system. Equilibrium may also not be hypothesized once the process of metal entering into the organism is limited by diffusional control across the (static) boundary layer surrounding the interfacial cells.

The basic assumption underlying the BLM (ion competition) potentially allows incorporating metal interactive effects into the assessment of mixture toxicity. In Table 1, some representative publications about the extended BLM for metal mixture assessment are summarized. At first, there is great interest in BLMs for metal mixtures in aquatic system. A multimetal modeling framework based on the BLM concept has been developed for aquatic organisms (Balistrieri et al., 2015; Farley & Meyer, 2015; Hatano & Shoji, 2008; Iwasaki et al., 2015; Playle, 2004; Santore & Ryan, 2015) and for microorganism (Jho et al., 2011; Liu et al., 2017). For terrestrial higher plants, attempts have been made to apply BLM concepts to analyze the combined effects of multiple metals in solution cultures (Le, Vijver, Jan Hendriks et al., 2013; Li et al., 2020; Liu et al., 2014; Qiu et al., 2015; Versieren et al., 2014; Wang et al., 2017). As shown in Table 1, the predictive capacity of each extended BLM method varied with different metal combinations, test species, or exposure mediums (soil or water), which may be due to the different underlying toxicity mechanisms of the different metals, differences in sensitivity, or other factors in the exposure regime. Qiu et al. (2016) successfully developed a multimetal soil BLM to explain the mixture effects and indicated that bioavailability factors dominated the interactive effects across soils. From that point on, the BLM framework was extended to soils for quantifying metal mixture toxicity. Recently, a BLM-Toxic Unit model has been successfully developed to quantify the toxicity of As-Se mixtures under the influence of varying anion exposures, explaining more than 77% of the observed variation in toxicity (Ji et al., 2020).

Table 1. Overview of the extended Biotic Ligand Model (BLM) fits to metal mixture toxicity data.

Metal mixtures	Test species	Exposure regime	Endpoints	Methods	R^{2a}	$RMSE^{b}$	References
Cu-Cd	duckweed, *Lemna paucicostata*	4-d, hydroponic exposure	growth rate	TU[c]	0.83	13.5	(Hatano & Shoji, 2008)
Cd-Pb	bacteria, *Vibrio fischeri*	hydroponic exposure	bioluminescence inhibition	TU	-[d]	25.6	(Jho et al., 2011)
				f_{mix}		9.7	
Cu-Ag	lettuce, *Lactuca sativa*	hydroponic exposure	RRE[e]	TEQ[f]	0.64	–	(Le, Vijver, Jan
				TEQ	0.69	–	Hendriks et al., 2013)
				TEQ	0.84	–	
Cu-Zn	lettuce, *Lactuca sativa*	hydroponic exposure	RRE	TEQ	0.65	–	(Le, Vijver, Jan Hendriks et al., 2013)
Cu-Ag	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	TU	0.86	10.5	(Liu et al., 2014)
				f_{mix}	0.58	18.5	
				TEF[g]	0.76	14.0	
Cu-Zn	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	TU	0.58	18.8	(Liu et al., 2014)
				f_{mix}	0.73	15.2	
				TEF	0.65	17.1	
Cu-Ni	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	TU	0.69	18.0	(Liu et al., 2014)
				f_{mix}	0.58	20.9	
				TEF	0.74	16.7	
Cu-Zn	barley, *Hordeum vulgare*	4-d, hydroponic exposure	RRE	f_{mix}	0.74	7.70	(Versieren et al., 2014)
Cu, Cd, Zn, Pb	trout, *Oncorhynchus mykiss* *Oncorhynchus clarki*	hydroponic exposure	mortality	acute fish models	> 0.73	–	(Balistrieri & Mebane, 2014)
Cu-Ni	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	f_{mix}	0.82	11.7	(Qiu et al., 2015)
Cu-Cd	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	f_{mix}	0.87	10.9	(Qiu et al., 2015)
Ni -Cd	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	f_{mix}	0.85	11.0	(Qiu et al., 2015)
Ag, Cd, Cu, Ni, Pb, Zn	*Hyalella azteca, Daphnia magna, Oncorhynchus mykiss*	hydroponic exposure	mortality	IA[h] TU	–	–	(Santore & Ryan, 2015)
Al-Cd-Cu-Ni-Pb-Zn	Hydropsychidae, *Arctopsyche grandis* Ephemerillidae, *Drunella doddsi* Heptageniidae, *Rhithrogenasp*	hydroponic exposure	mortality	'chronic' field invertebrate models	0.65	–	(Balistrieri et al., 2015)
Cd-Pb-Zn	rainbow trout, *Oncorhynchus mykiss*	hydroponic exposure	mortality	f_{mix}	0.82	0.28	(Iwasaki et al., 2015)
Cu-Cd	zebrafish, *Danio rerio* AB strain	24h, hydroponic exposure	mortality	f_{mix}	0.81	–	(Gao, Feng, & Zhu, 2016)
				TEQ	0.82	–	
Cu-Pb	zebrafish, *Danio rerio* AB strain	24h, hydroponic exposure	mortality	f_{mix}	0.76	–	(Gao, Feng, & Zhu, 2016)
				TEQ	0.77	–	
Cd-Pb	zebrafish, *Danio rerio* AB strain	24h, hydroponic exposure	mortality	f_{mix}	0.62	–	(Gao, Feng, & Zhu, 2016)
				TEQ	0.91	–	
Cu-Zn	barley, *Hordeum vulgare*	5-d, soil experiments	RRE	f_{mix}	0.77	16.1	(Qiu et al., 2016)
Cu-Zn	bacterium, SD5	hydroponic exposure	relative nitrification rate	f_{mix}	0.89	19.7	(Liu et al., 2017)
				TU	0.69	31.1	
Cu-Co	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	f_{mix}-TU	0.97	6.70	(Wu et al. 2016)
La-Ce	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	CA[i]	0.92	8.56	(Li et al., 2020)
As-Se	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	f_{mix}-TU	0.77	9.30	(Ji et al., 2020)

[a] R^2 indicates the goodness of fit. [b] *RMSE* represents the root-mean-squared error. [c] TU = toxic unit. [d] - indicates unknown value. [e] RRE = relative root elongation. [f] TEQ = toxic equivalent. [g] TEF = toxic equivalency factor. [h] IA = Independent Action. [i] CA = Concentration Addition.

This is the first systematic study on the single and mixture toxicity of anionic metal(loid)s under the influences of varying anion concentrations.

2.1.3. Main advantages and disadvantages in predicting toxicity of metal mixtures

The BLM-based model considers specific ion-ion interactions by including the assumption of competitive or noncompetitive binding. Thus, ion-ion interactions of metal mixtures are only interpreted by competition for BLs in this approach. Besides, influences of cations are not always integrated as the effect of cations is technically considered in the BLM only when a linear significant relationship is observed between the response of organisms and the cation exposure concentration. Until now, most of the BLM-based models for metal mixtures are based on the parameters derived from individual metal toxicity data. Ideally, it is fairly straightforward to construct such a model to predict metal mixture toxicity if the binding constants of metal ions as well as other coexisting cations for the BLs are available. It should be noted that to derive the binding constants of interest often requires large univariate toxicity data sets. Experimental and modeling uncertainties may induce variations of the obtained parameters, and thus calibration is required for each metal and species.

2.1.4. Future perspectives

It is undeniable that the BLM framework has a great potential for the quantitative modeling of the mixture toxicity of metal(loid)s in hydroponic or soil systems because its theoretical basis is widely accepted for most organisms. However, extended BLM models should be further improved for interpreting synergistic interactions between metal mixtures. For risk assessments of contamination with metal mixtures in the field, further research is required to evaluate metal mixture toxicity in a wide range of field soils. Based on the fact that both cationic and anionic metal(loid)s are ubiquitous in the environment, further research efforts are required to refine the BLM framework so that the interactive effects between cations and anions can be considered simultaneously within a model framework.

2.2. Electrostatic toxicity model (ETM)

2.2.1. Basic concepts and principles

Based on the fact that the root plasma membrane (PM) surface expresses negative charges which result in obvious differences between ion concentrations in the bulk medium and at the PM surface, the ETM has been developed to explain how a corresponding electrical potential (φ_0) at the PM

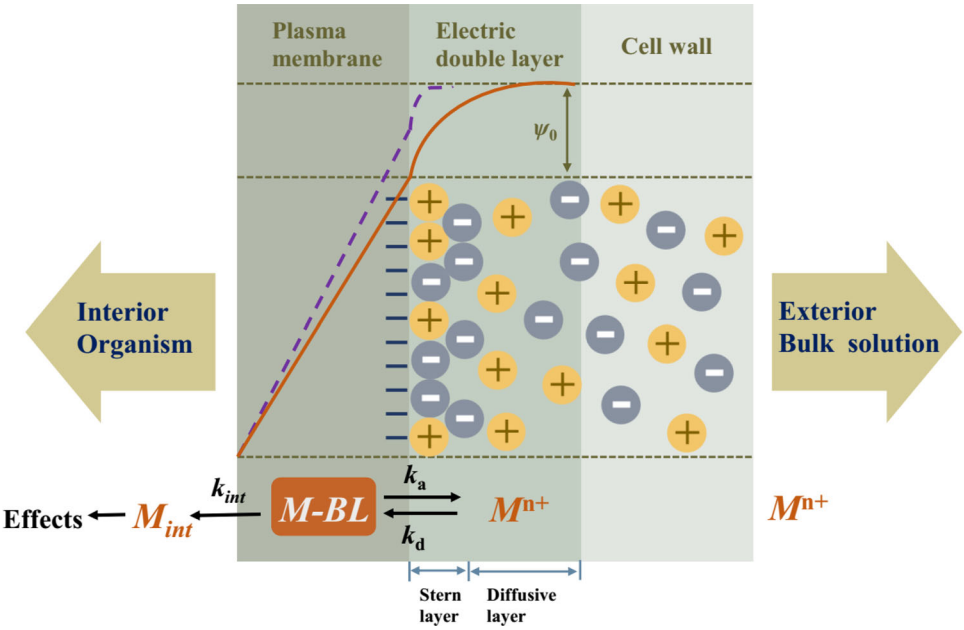

Figure 2. Brief description of the Electrostatic Toxicity Model (ETM). Metal ion (M^{n+}) transport across the plasma membrane (PM), including dissociation (k_d)/association (k_a) with the active binding sites on the surface of the PM (M-BL) and internalization (k_{int}) into the cell interior.

surface affects plant-ion interactive effects (Figure 2) (Kinraide, 1998; Wagatsuma & Akiba, 1989). The φ_0, which is generally negative, could influence the free ion activities at the PM surface by attracting cations or repelling anions from the bulk medium, and meanwhile provide the electrical driving force for ion transport across the cell membrane (Kinraide, 2001; Kopittke, Blamey et al., 2011). Therefore, the addition of cations in the bulk medium (such as Ca^{2+}, Mg^{2+}, Al^{3+}, H^{+}) could reduce the negativity of φ_0 in a nonspecific manner thereby enhancing the toxicity of other anions (such as SeO_4^{2-}) (Kinraide, 2010a) or alleviating toxicity of cations (such as Ni^{2+}, Cu^{2+}, Zn^{2+}) (Kinraide, 1999; Kopittke, Kinraide et al., 2011; Le & Peijnenburg, 2017; Wang, Kinraide et al., 2011; Wang, Kopittke et al., 2011). Taking into account the φ_0 at the PM surface, the ETM could be well adapted to many cases where metal bioavailability could not be entirely explained by site-specific competitions (Kinraide, 2010b). The value of φ_0 could be determined from the specific ionic composition of the bulk solution using a Gouy-Chapman-Stern (GCS) model (Kinraide, 1998), somehow involving ion-ion interactive effects. Kinraide (2010b) also proposed simplified methods to easily obtain this value according to available literature. For example, equilibrium constants could be estimated by a formula according to the "Hard Ligand Scale" and thus be used to calculate φ_0 (Kinraide & Yermiyahu, 2007). A computer program is available for the determination of φ_0 and PM ion activities (Kopittke et al., 2014).

Some investigations have shown that bulk solution chemistry was inadequate to predict the bioavailability of metals to organisms due to different conditions near the cell membrane (Liu et al., 2018; Sánchez-Marín et al., 2018). Many studies have proven that $\{M^{z+}\}_{surf}$, the free ion activity at the PM surface calculated from φ_0, is a better predictor of metal phytotoxicity than that in the bulk medium (donated as $\{M^{z+}\}_{bulk}$) (Gong et al., 2019). The estimation of $\{M^{z+}\}_{surf}$ via $\{M^{z+}\}_{bulk}$ and φ_0 can be realized by the Nernst Equation:

$$\{M^{z+}\}_{surf} = \{M^{z+}\}_{bulk} \times \exp\left[-\frac{Z \times F \times \varphi_0}{RT}\right] \quad (4)$$

where $\{M^{z+}\}_{surf}$ and $\{M^{z+}\}_{bulk}$ are free M^{z+} ion activities at the PM surface and in the bulk solution, respectively; Z is the charge of the metal ion; F the Faraday constant; R the universal gas constant; and T the experimental temperature.

2.2.2. Application in assessment of metal toxicity

The applicability of the electrostatic theory has been evaluated in many studies for the prediction of individual metal toxicity (Gong et al., 2019; Kopittke, Blamey et al., 2011; Wang, Kinraide et al., 2014; Zhou & Wang, 2011). As shown in Table 2, the toxicity of metals such as Ni^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Y^{3+}, and Ce^{3+} to several plant species, including *Triticum aestivum* (wheat), *Hordeum vulgare* (barley) and *Vigna unguiculata* (cowpea), as well as impacts of cations such as Ca^{2+}, Mg^{2+}, and H^+ could be well predicted by the ETM ($R^2 > 0.77$). Gong et al. (2019) reported that the predictive capacity of the ETM was nearly equal to that of BLM in quantifying the toxicity of Y and Ce to wheat in hydroponic culture. Indeed, the electrostatic theory has been proposed as a surrogate to the BLM in modeling metal bioavailability, uptake, and toxicity (Kopittke, Blamey et al., 2011; Wang et al., 2008). Wang, Kopittke et al. (2011) demonstrated that φ_0 played dual roles in the toxicity of Ni^{2+} to barley in both hydroponic and soil cultures. Firstly, the reduced negativity of φ_0 due to the addition of cations such as Ca^{2+}, Mg^{2+} and H^+ decreased the Ni^{2+} activity at the PM surface (Kinraide, 2001). Secondly, with the addition of cations, φ_0 was likely to increase the surface-to-surface transmembrane potential difference, which is the electrical driving force for Ni uptake across membranes. Therefore, the toxicity of Ni was a sum of the two relatively opposite effects induced by membrane surface potential φ_0 (Wang, Kopittke et al., 2011).

Only recently has the ETM been extended to estimate metal mixture toxicity (Le et al., 2014; Li et al., 2020; Qiu & He, 2017; Wang, Wang et al., 2014; Wang, Zhou et al., 2018; Wang et al., 2013). Most of these available publications focused on the applications of ETM in simplified hydroponic

Table 2. Overview of the Electrostatic Toxicity Model (ETM) fits to toxicity data for individual metals and metal mixtures.

Metals	Test species	Exposure regime	Endpoints	Methods	R^{2a}	$RMSE^{b}$	References
Ni	barley, *Hordeum vulgare*	4-d, hydroponic exposure	RRE[c]		0.77	18.0	(Wang, Kopittke et al. 2011)
		5-d, hydroponic exposure	RRE		0.92	9.10	
		21-d, soil experiments	RRE		0.94	9.74	
Pb	cowpea, *Vigna unguiculata*	48-h, hydroponic exposure	RRE		0.97	-[d]	(Kopittke, Kinraide et al., 2011)
Cu	wheat, *Triticum aestivum*	2-d, hydroponic exposure	RRE		0.92	–	(Zhou & Wang, 2011)
Zn	wheat, *Triticum aestivum*	48-h, hydroponic exposure	RRE		0.91	–	(Wang, Kinraide et al., 2014)
Y	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE		0.92	9.46	(Gong et al., 2019)
Ce	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE		0.87	11.8	(Gong et al., 2019)
Zn-Co	wheat, *Triticum aestivum*	48-h, hydroponic exposure	RRE	IA[e]	0.93	–	(Wang et al., 2013)
Zn-Co	wheat, *Triticum aestivum*	48-h, hydroponic exposure	RRE	CA[f]	0.81	–	(Wang, Zhou et al., 2014)
				IA	0.82	–	
Cu-Zn	lettuce, *L. sativa*	4-d, hydroponic exposure	net root growth	CA	0.92	–	(Le et al., 2014)
Cu-Ag	lettuce, *L. sativa*	4-d, hydroponic exposure	net root growth	CA	0.80	–	(Le et al., 2014)
Cu-Zn	lettuce, *L. sativa*	4-d, hydroponic exposure	net root growth	TU[g]	0.58	17.9	(Le & Peijnenburg, 2017)
Cu-Ag	lettuce, *L. sativa*	4-d, hydroponic exposure	net root growth	TU	0.61	20.5	(Le & Peijnenburg, 2017)
Cu-Zn	barley, *Hordeum vulgare*	5-d, soil experiments	RRE	CA	0.89	11.7	(Qiu & He, 2017)
Cu-Cd	wheat, *Triticum aestivum*	48-h, hydroponic exposure	RRE	CA	0.93	–	(Wang, Zhou et al., 2018)
La-Ce	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	CA	0.90	9.64	(Li et al., 2020)

[a]R^2 indicates the goodness of fit. [b]*RMSE* represents the root-mean-squared error. [c]RRE = relative root elongation. [d]- indicates unknown value. [e]IA = Independent Action. [f]CA = Concentration Addition. [g]TU = toxic unit.

cultures. Qiu and He (2017) first investigated the applicability of the ETM for predicting the uptake and toxicity of Cu-Zn mixtures to *Hordeum vulgare* in different soils. The authors reported that this approach was theoretically and empirically feasible in evaluating metal mixture toxicity in soils. In these publications, free ion activities of single metals at the PM surface calculated from φ_0 were regarded as excellent predictors of mixture toxicity by the conventional CA or IA model, and ultimately can be used to identify the different interactive effects types, such as additivity, synergism and antagonism (Wang et al., 2013). As shown in Table 2, a few publications have evaluated the performance of the ETM in predicting the toxicity of mixtures of metals, such as Zn-Co, Cu-Cd, and Cu-Zn, to several plant species. Interactive effects between metals at different levels can be incorporated into the ETM, which contribute to a better understanding of metal mixture toxicity (Le et al., 2014; Wang, Kinraide et al., 2014). Specifically, interactive effects between metal ions occurring at the near outside of the surface can be represented by the changes in the free metal ion activity at the PM surface with varying activities of another one in the bulk medium. In addition, internal interactive effects can be incorporated into mixture toxicity models to predict and interpret the toxicity of metal mixtures based on free ion activities at the PM surface (Le & Peijnenburg, 2017).

2.2.3. Main advantages and disadvantages in predicting toxicity of metal mixtures

The ETM has the capacity of taking into account electrostatic interactions. It can explain effects of all components in the exposure medium. This approach has the advantage of simply obtaining the required modeling parameters and thus greatly simplify the process for quantifying metal mixture toxicity. However, only the statistically significant fitting parameters will be included in this model, which may miss its biological meaning. Notably, the electrostatic theory ignores specific binding at discrete sites and cannot account for ion-ion interactions. In addition, changes of φ_0 at the PM surface do not necessarily reflect the interactions between metals with the similar physicochemical characteristics.

2.2.4. Future perspectives

On the basis of these successful applications, the use of the ETM to predict metal mixture toxicity is simple and robust in both water and soil. At present, this conclusion is only effective for short-term metal toxicity of mixtures, and the suitability for dealing with long-term metal mixture toxicity remains unclear. Furthermore, this tool can be readily implemented in assessing site-specific risks using chemical analysis data of the site of

interest. Further investigations on different types of soils based on different metal mixture combinations as well as different test species would be meaningful to construct a solid basis for incorporating this approach into risk assessment of contamination by metal mixtures in soil.

2.3. WHAM-F_{tox} approach

2.3.1. Basic concepts and principles

This promising mechanistic-underpinned bioavailability model relating biological responses to chemical speciation has gained increasing attention. The assumption of the WHAM-F_{tox} model is that the amount of exposure to metals is proportional to metals binding with weak-acid coordination sites on/in the living organisms, in equilibrium with the ambient medium (Tipping et al., 2019). The bioavailability of metals can be reflected by the fractional occupancy of binding sites, which is similar to the measure of contamination using metal body burdens (Borgmann et al., 2008; Wang, 2013). In this model, it is postulated that cation binding sites of organisms can be represented by the particulate humic acid (HA) (Figure 3) (Stockdale et al., 2014). Particularly, HA contains many functional groups, which represent the heterogeneous distribution of BLs.

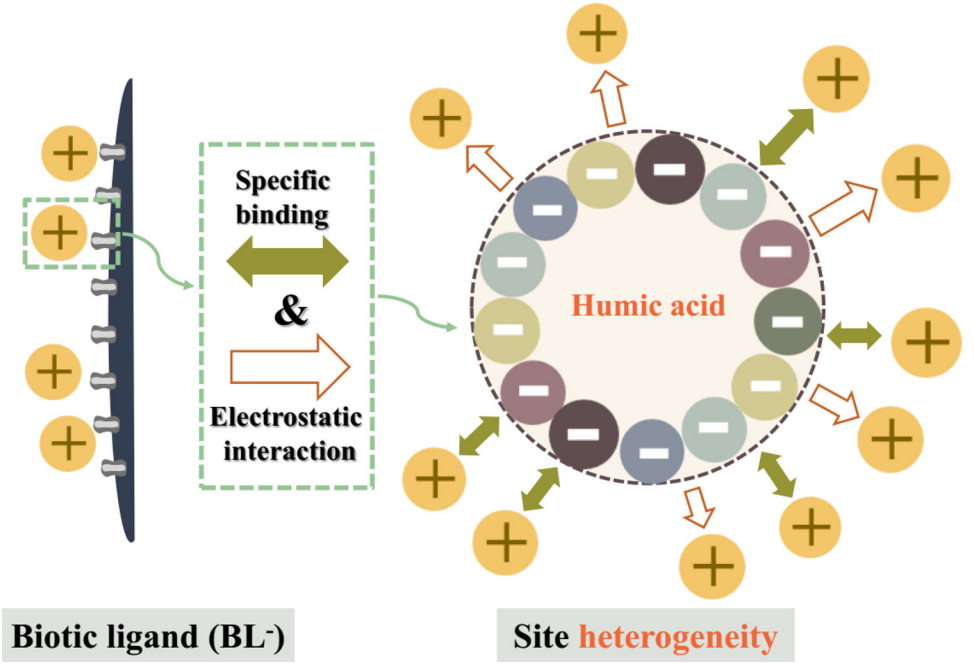

Figure 3. Brief description of the WHAM-F_{tox} approach. Minus circles with different colors represent the different functional groups on the surface of the humic acid. Metal cations denoted as plus circles might bind with these different functional groups.

This model quantifies the mixture toxicity of metal ions and protons toward organisms with a linear toxicity function F_{tox} (mol/g), which is the sum of the products of organism-bound cations and toxicity coefficients (Tipping & Lofts, 2013):

$$F_{tox} = \sum \alpha_i v_i \tag{5}$$

where ν_i (mol/g) is the concentration of metal ions or proton bound to particulate HA, which is calculated by the Windermere humic aqueous model (WHAM VII), and α_i (dimensionless) represents the toxicity coefficient of the metal or proton.

The concentrations of metals binding to particulate HA can easily be calculated by WHAM VII. A database of particulate HA is available in WHAM VII, thus facilitating the acquisition of the parameters needed for the WHAM-F_{tox} approach. The particulate HA was incorporated into all the speciation computations and its concentration was assigned at a sufficiently low level (5.0×10^{-6} g/L) to avoid affecting metal speciation in WHAM VII to calculate ν_i (Stockdale et al., 2010). It should be noted that only the relative values of ν_i rather than its absolute values are of interest in this approach. The toxic response R depends on a threshold model according to the following definitions:

$$R = \begin{cases} 100 & F_{tox} \le F_{tox-min} \\ \dfrac{F_{tox-max} - F_{tox}}{F_{tox-max} - F_{tox-min}} \times 100 & F_{tox-max} > F_{tox} > F_{tox-min} \\ 0 & F_{tox} > F_{tox-max} \end{cases} \tag{6}$$

where $F_{tox\text{-}min}$ is a lower threshold of F_{tox}, less than which there is no toxic effect; $F_{tox\text{-}max}$ is an upper threshold of F_{tox}, more than which a maximum toxic effect occurs. For values of F_{tox} in between, the toxic response is hypothesized to alter linearly with its value.

2.3.2. Application in assessment of metal mixture toxicity

As shown in Table 3, many efforts have recently been put into applying the WHAM-F_{tox} approach for assessing metal bioavailability and toxicity in mixture scenarios. One aspect of F_{tox} that should be noteworthy is that its origin was in predicting aquatic insect communities in field data (Stockdale et al., 2010), and subsequently was successfully applied to lab data. Most models are developed the other way around. This approach is not only allowed for aquatic organisms but can be further extended to higher organisms (e.g., plants and prokaryotic) (Tipping & Lofts, 2015). Lately, the WHAM-F_{tox} has been demonstrated to be a promising tool to

Table 3. Overview of the WHAM-F_{tox} model fits to different metal mixture toxicity data.

Metal mixtures	Test species	Exposure regime	Endpoints	R^{2a}	$RMSE^b$/ $RMSD^c$	References
Al-Ni-Cu-Zn-Cd-Pb	Invertebrate community	streamwater	species richness	> 0.73	-[d]	(Stockdale et al., 2010)
Cu-Zn, Cu-Cd, Cd-Zn	bacteria, *Escherichia coli*	15-m, hydroponic exposure	luminescence inhibition	0.68	19.0	(Tipping & Lofts, 2013)
Cu-Zn, Cu-Cd, Cd-Zn	bacteria, *Pseudomonas fluorescens*	15-m, hydroponic exposure	luminescence inhibition	0.89	10.0	(Tipping & Lofts, 2013)
Cd-Pb	bacteria, *Vibrio fischeri*	5-m, hydroponic exposure	luminescence inhibition	0.81	15.0	(Tipping & Lofts, 2013)
Cu-UO_2	duckweed, *Lemna aequinoctialis*	96-h, hydroponic exposure	growth rate	0.96	8.00	(Tipping & Lofts, 2013)
Cu-Cd	duckweed, *Lemna paucicostata*	96-h, hydroponic exposure	growth rate	0.76	18.0	(Tipping & Lofts, 2013)
Zn-Cd	daphnid, *Ceriodaphnia dubia*	96-h, hydroponic exposure	survival	0.77	16.0	(Tipping & Lofts, 2013)
Zn-Cd	daphnid, *Daphnia ambigua*	96-h, hydroponic exposure	survival	0.96	7.00	(Tipping & Lofts, 2013)
Zn-Cd	daphnid, *Daphnia magna*	96-h, hydroponic exposure	survival	0.84	14.0	(Tipping & Lofts, 2013)
Zn-Cd	daphnid, *Daphnia pulex*	96-h, hydroponic exposure	survival	0.88	11.0	(Tipping & Lofts, 2013)
Cu-Zn, Zn-Cd, Cu-Cd, Cu-Zn-Cd	mussel, *Dreissena polymorpha*	48-h, hydroponic exposure	filtration rate	0.92	11.0	(Tipping & Lofts, 2013)
Al-Cu-Zn	trout, *Oncorhynchus mykiss*	144-h, hydroponic exposure	survival	–	5.00	(Tipping & Lofts, 2013)
Cu-Ni	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE[e]	0.79	13.0	(Qiu et al., 2015)
Cu-Cd	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	0.87	10.9	(Qiu et al., 2015)
Ni -Cd	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	0.81	12.7	(Qiu et al., 2015)
Co-Zn	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	0.88	13.1	(Qiu et al., 2015)
Cu-Al	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	0.86	12.9	(Qiu et al., 2015)
Cu-Mn	lettuce, *Lactuca sativa*	4-d, hydroponic exposure	RRE	0.70	17.7	(Qiu et al., 2015)
Ni -Co	Oligochaeta, *Enchytraeus crypticus*	4, 7, 10 and 14-d, hydroponic exposure, inert quartz sand	survival	> 0.79	–	(He & Van Gestel, 2015)
Cd-Cu-Zn	daphnid, *Daphnia magna*	hydroponic exposure	survival	0.66	–	(Balistrieri et al., 2015
Al-Cd-Cu-Ni-Pb-Zn	Hydropsychidae, *Arctopsyche grandis* Ephemerillidae, *Drunella doddsi* Heptageniidae, Rhithrogenasp	hydroponic exposure	mortality	0.65	–	(Balistrieri et al., 2015)
Al-Cu-Ni-Zn	zooplankton	hydroponic exposure	taxa poorness	0.74	–	(Balistrieri et al., 2015)
Cu-Zn, Cu-Ag	lettuce, *Lactuca sativa*	hydroponic exposure	RRE	0.78	14.0	(Tipping & Lofts, 2015)
Zn-Pb, Zn-Cd, Zn-Cd-Pb	trout, *Oncorhynchus clarkii lewisi*	hydroponic exposure	survival	0.81	17.0	(Tipping & Lofts, 2015)
Zn-Pb, Zn-Cd, Zn-Cd-Pb	trout, *Oncorhynchus mykiss*	hydroponic exposure	survival	0.64	24.0	(Tipping & Lofts, 2015)
Cu-Zn	barley, *Hordeum vulgare*	5-d, soil experiments	RRE	0.83	13.3	(Qiu et al., 2016)
Cu-Zn	lettuce, *Lactuca. sativa*	hydroponic exposure	RRE	0.66	15.9	(Le & Peijnenburg, 2017)
Cu-Ag	lettuce, *Lactuca sativa*	hydroponic exposure	RRE	0.71	17.6	(Le & Peijnenburg, 2017)
Y-La-Ce	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	0.85	–	(He et al. 2020)
Y-La, Y-Ce, La-Ce	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	0.88	–	(He et al. 2020)
La-Ce	wheat, *Triticum aestivum*	4-d, hydroponic exposure	RRE	0.88	8.93	unpublished data

[a]R^2 indicates the goodness of fit. [b]*RMSE* represents the root-mean-squared error. [c]*RMSD* represents the root-mean-squared deviation. [d]- indicates unknown value. [e]RRE = relative root elongation.

account for the interactive effects of rare earth elements in binary and ternary mixtures with more than 88% and 85% of the variation in toxicity explained, respectively (He et al., 2020). For most metal mixture toxicity data in Table 3, the predictive capacities of the WHAM-F_{tox} approach in describing the toxicity of different metal mixtures were comparable to classic conceptual models and the extended BLM model. For example, Qiu et al. (2016) demonstrated that the capacity of the WHAM-F_{tox} was comparable to the extended BLM model in normalizing interactions and toxicity of Cu-Zn mixtures to barley in different soils. This was the first attempt to apply this model to predict mixture toxicity of metals in different soils. In addition, Balistrieri et al. (2015) applied F_{tox} to lake zooplankton community data and stream invertebrate datasets with good results. The authors tested the F_{tox} model against two BLM models and found that all three approaches were successful at modeling accumulation of metals in insects and effects to the overall communities. Depending on insect species and metal, correlation coefficients (r^2) between measured metal accumulation and model predictions ranged from 0.01 to 0.80 for F_{tox} "out of the box", versus 0.00 to 0.86 for the two BLMs that had their parameters specifically fit to that dataset. The fact that the simple F_{tox} model performed very well with data it has never "seen" before speaks well to its utility and versatility.

One important feature/limitation of F_{tox} is that organisms are not just little bags of HA swimming around in the water. At least Ag, Cu, Cd, and Zn are believed to be taken up by active transport via Na or Ca channels, which means their effective affinity to BLs is much higher than that of HA. Therefore, it is not surprising that the capacity of the WHAM-F_{tox} approach in predicting Cu-Ag toxicity to *L. sativa* was poor (Qiu et al., 2015). Interestingly, Le, Vijver, Jan Hendriks et al. (2013) reported that a multimetal BLM failed in delineating Cu-Ag toxicity to lettuce with only 64% of the variance in toxicity explained. Taking together, neither the WHAM-F_{tox} approach nor the BLM can be used to predict Cu-Ag toxicity. The assumption of the WHAM-F_{tox} approach is that competitive interactive effects between metals and protons take place at the reversible binding sites (Tipping & Lofts, 2015). The possible explanation for this special case is that the competition hypothesis may not apply to Cu-Ag mixtures, which suggests that Cu^{2+} and Ag^{+} are conveyed into organisms through different transporters (Le, Vijver, Jan Hendriks et al., 2013). Experimental uncertainties, simplification of models together with interactive effects of metals at the internal level may be reasons why still part of the variation in toxicity could not be explained.

2.3.3. Main advantages and disadvantages in predicting toxicity of metal mixtures

The WHAM-F_{tox} approach is effective to delineate synergistic or antagonistic interactions of metal mixtures. Like ETM, this model is advantageous for handling lower data availability based on an available WHAM database of cations reversibly binding to nonspecific BLs. Consequently, it has the potential to be extended for different species and metal mixture combinations. Meanwhile, adjustments are thus required for toxicological parameters in different scenarios. As discussed above, the assumption that competitive chemical reactions can be represented by competitive binding to particulate HA needs further validation. The simplification of the model relying on stepwise multiple linear regression analysis will likely miss its biological meaning.

2.3.4. Future perspectives

The mechanistic-based WHAM-F_{tox} approach is superior in predicting metal mixture toxicity in different exposure media. Its applicability to different test species, to chronic toxicity data, or to real contaminated soils with varying properties needs to be further investigated.

3. Biodynamic and toxicokinetic-toxicodynamic model (TK-TD)

Previous toxicological studies on metal mixtures focused on the toxicity at fixed exposure duration and constant external exposure levels. However, the accumulation of metals in organisms is time dependent, resulting in effects that vary with exposure time (Baas et al., 2007). Consequently, related kinetic modeling approaches are needed to accurately predict metal accumulation and toxicity under various environmental conditions. Adams et al. (2011) summarized and reviewed some biodynamic approaches for assessing metal accumulation and effects. Buchwalter et al. (2007) demonstrated that biodynamic modeling was a promising tool to better understand interspecific differences in metal bioaccumulation. The authors proposed an important concept relating to the subcellular partitioning of accumulated metals in sensitive taxa. In addition, Rainbow (2002) reported that toxicity was related to the internal threshold concentration of a metabolically available pool rather than to the total accumulated metal concentration. They proposed a concept of metabolically reactive metal that could be separated from stored and detoxified metal by subcellular fractionation techniques. These biodynamic approaches associated with mechanistic concepts would increase a realistic understanding of metal toxicity.

3.1. Basic concepts and principles

A Toxicokinetic-toxicodynamic (TK-TD) model has been further developed to help in the description of the dynamic accumulation and toxicity of

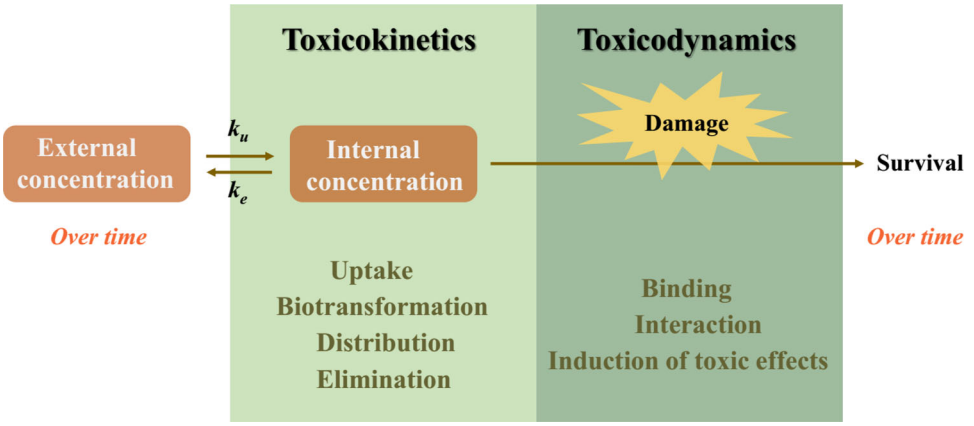

Figure 4. Brief description of the Toxicokinetic-Toxicodynamic (TK-TD) Model. TK is concerned with what the living organism does to the toxicant; TD is concerned with what the toxicant does to the living organism. k_u = the uptake rate constant (L g^{-1} d^{-1}), k_e = the efflux rate constant (d^{-1}).

metals (He & Van Gestel, 2013). This model is suitable for assessing toxicokinetics (external to internal concentration of toxic substances) and toxicodynamics (internal concentration over time to effects of toxic substances) based on mechanisms of toxicity (Figure 4). Toxic substances first need to be taken up and transported to the target or active site before they can exert effects at the organism level. TK models can translate the external concentration into an internal concentration as a function of time. TK models can be divided into compartmental TK models and physiologically based pharmacokinetic (PBPK) models. The compartmental TK models can be divided into one-compartment and two-compartment models. When metals are regarded in the organism as a whole, a first order one-compartment kinetic model as its simplest form can be used (Stadnicka-Michalak et al., 2014):

$$\frac{dC_i(t)}{dt} = k_u C_w(t) - k_e C_i(t) \tag{7}$$

where $C_i(t)$ is the internal metal concentration over time, $C_w(t)$ is the time-course of metal exposure concentration. The parameters k_u and k_e are the uptake and efflux rate constants, respectively. If the saturation of the metal uptake rate is considered, k_u can be rewritten as follows using a Michaelis-Menten equation:

$$ku = \frac{J_{M,\ max}}{K_m + C_w(t)} \tag{8}$$

where $J_{M,max}$ is the maximum metal (M) uptake rate, K_m the Michaelis–Menten constant or the concentration at which transport sites are half saturated.

The two-compartment model mainly distinguishes metabolically available and detoxified metal fractions inside organisms (Rainbow & Luoma, 2011):

$$C_i(t) = C_1(t) + C_2(t) \tag{9}$$

$$\frac{dC_1(t)}{dt} = ku C_w(t) - (k_{e1} + k_{12}) \times C_1(t) + k_{21} \times C_2(t) \tag{10}$$

$$\frac{dC_2(t)}{dt} = k_{12} \times C_1(t) - k_{21} \times C_2(t) \tag{11}$$

where $C_1(t)$ and $C_2(t)$ are the metabolically available and detoxified metal concentrations over time, respectively. The parameter k_{e1} is the efflux rate constant of the metabolically available metal, and k_{12} and k_{21} are the metal transfer rate constants from the metabolically available to detoxified metal and from detoxified to the metabolically available metal, respectively. If we consider saturation of the metal uptake rate, k_u can be rewritten as Eq. 8.

Historically, the PBPK concept is proposed mainly in pharmacological research in order to predict drug transport and metabolism within different organs (Gerlowski & Jain, 1983). This in fact is a multicompartment model, which can be used to describe the absorption, distribution, metabolism and excretion of toxic substances among multiple tissues in an organism. It divides organisms into compartments of real tissues or organs connected by fluid (usually blood). The structure of the PBPK model depends largely on the purpose of developing the model and whether enough toxicity data can be obtained. Choosing a model to keep its structure as simple as possible is the first guiding principle.

Concentrations of toxic substances at target or active sites may not be sufficient to explain the dynamic process of toxicity over time. Therefore, the concept of "damage" is introduced in the TD model (Jager et al., 2011). The internal concentration of toxic substances in an organism causes damage to the organism, which is repaired at a certain rate. Furthermore, the quantitative relationship between the degree of damage and the endpoint of effect at the individual level can be established. The two basic assumptions in the TD model are individual tolerance (IT) and stochastic death (SD) (Jager et al., 2011). Assuming that individuals have a different sensitivity to toxic substances and individuals dying are more sensitive than surviving ones at a certain point in time, IT is suitably selected (Nyman et al., 2012):

$$F(t) = \frac{1}{1 + \left(\max C_i^*(\tau)/LC50\right)^{-\alpha}} (0 < \tau < t) \tag{12}$$

where $F(t)$ is the log-logistic cumulative distribution function for the threshold, $\max C_i^*$ is the maximum internal concentration ever reached from time 0 to t, $LC50$ is the median of the distribution, α determines the width of the distribution.

The survivorship function is written as Eq. 13:

$$S(t) = (1 - F(t)) \times \mathrm{e}^{-\mathrm{h}_0 \times \mathrm{t}} \tag{13}$$

where $S(t)$ is the survival probability of the organism, h_0 is the control hazard rate.

SD assumes that the biological death caused by toxic stress is a random process, that is, every individual has the same chance of dying, and this chance increases with the increase of exposure to stressors. $S(t)$ is written as follows (Jager et al., 2011):

$$\begin{aligned} &\frac{dH(t)}{dt} = k_k(C_1(t) - C_{1\mathrm{T}}) + h_0, \text{ if } C_1(t) > C_{1\mathrm{T}} \text{ else } \frac{dH(t)}{dt} = h_0; \\ &S(t) = e^{-H(t)}; \\ &S_0(t) = e^{-h_0 \times t} \end{aligned} \tag{14-16}$$

where $H(t)$ is the hazard, k_k is the killing rate constant, C_{IT} defines the boundary between safe and toxicity, $S_0(t)$ is the control survival probability of organisms.

In case of investigating the relationship between exposure time and toxic effects, the Critical Body Residue (CBR) model is most prominent (Borgmann et al., 2008; Wen et al., 2015). This model postulates that a living organism will die if its internal threshold concentration is exceeded. In this concept, the relationship between biokinetics and toxicity is taken into account, which is applicable for compounds that react reversibly with the specific receptors, such as narcotic chemicals (Mackay et al., 1992). The basic assumption of the CBR model is that toxicity is determined by the time course of the internal concentrations. Adams et al. (2011) critically reviewed the CBR concept including the various iterations of the biodynamics approach. However, Vijver et al. (2004) gave a broader review, which was relevant to the problem of CBR not working very well as a predictor of toxicity. There is nothing to prevent it from extending to the Critical Target Occupation (CTO) model (Legierse et al., 1999), where mortality is postulated to take place if compounds irreversibly occupy a critical number of targets. Subsequently, the concept of CBR has been further developed into the PULSETOX model (Reinert et al., 2002) and the acute toxicity model of DEBtox (Péry et al., 2002; 2003), where the toxicity is assumed to be proportional to the concentration of the compound beyond the internal no-effect concentration (NEC) within the organism. The breakthrough in the development of TK-TD models is introducing a state variable for damage, which describes the changes of system properties over time (Ankley et al., 1995). The TD model based on damage variables has been further developed into a Damage Assessment Model (DAM) (Lee et al., 2002), which assumes that there is a probability distribution of

individual tolerance (Zhao & Newman, 2007). Most current TK-TD models are developed based on general unified threshold model of survival (GUTS) (Jager et al., 2011). This provides a conceptual framework to facilitate the use of different dose descriptors (external concentration, internal concentration, or damage) in the model (Ashauer et al., 2016).

3.2. Application in assessment of metal toxicity

In recent years, the TK-TD model has been successfully applied to simulate and predict toxicity over time (Cedergreen et al., 2017; Jager et al., 2011). Compared with the traditional dose-response analysis, the TK-TD model provides an alternative angle for toxicity assessment with more mechanistic and biological relevance by considering bioaccumulation processes of metals and corresponding toxicity during the time of exposure. However, the application of the TK-TD model to evaluate metal mixture toxicity is just beginning (Table 4). Wang, Liu et al. (2018) successfully developed a multimetal interactive effect model (TK process) to predict the toxicity of multimetals to *Daphnia magna* based on kinetic processes and internal interactive effects. A TK model was also developed to predict the accumulation of metal mixtures with additive or antagonistic effects in zebrafish larvae based on parameters derived from single metal exposures (Gao et al., 2018). The TK model was successfully used to simulate and predict the

Table 4. Overview of the Toxicokinetic-Toxicodynamic model (TK-TD) fits to different metal mixture toxicity data.

Metal mixtures	Test species	Model used	Notes	References
Cd-Pb, Cu-Cd, Cu-Pb	zebrafish, *Danio rerio*	TK-TD aided with BLM and toxic equivalent factor (TEF)	The accumulation and toxicity of metal mixtures were accurately predicted by applying a refined TK-TD model.	(Gao, Feng, Han et al., 2016)
U-Cd	nematode, *Caenorhabditis elegans*	DEBtox -integrated CA and IA	The joint toxicity of U and Cd was overestimated using the DEBtox framework.	(Margerit et al., 2016)
Cu-Zn Cu-Cd Cu-Pb Cd-Pb	zebrafish, *Danio rerio*	TK integrated CA and IA	CA and IA models showed consistent interactions patterns of metal mixtures in the TD process.	(Gao et al., 2018)
Ni-Cu-Zn	oyster, *Crassostrea hongkongensis*	one-compartment TK	TK model was effective for simulating the metal bioaccumulation in a complex and dynamic environment.	(Tan et al., 2018)
Pb-Cd-Cu-Zn	daphnid, *Daphnia magna*	multimetal interaction	Metal mixtures were analyzed through a combination of kinetic process and internal interactions.	(Wang, Liu et al., 2018)

time-course of multiple metal bioaccumulation in the oyster *Crassostrea hongkongensis* in a dynamic estuary polluted by metals (Tan et al., 2018). Based on the dynamic energy budget theory, the TK-TD model was developed to predict the toxicity of metal mixtures to *Caenorhabditis elegans* (Jager et al., 2014; Margerit et al., 2016). The TK-TD model could well simulate and predict the accumulation but not the toxicity of metal mixtures (Gao, Feng, Han et al., 2016). Croteau and Luoma (2009) and Balistrieri et al. (2020) applied biodynamic models to predict both accumulation and toxicity of metal mixtures in snails and stream insect communities, respectively. In the latter study, the authors linked equilibrium, biodynamic, and toxicity functions that evaluate metal mixture toxicity to aquatic insect families. Their modeling indicated that Cd, Cu, and Ni but not Co and Zn were major contributors to the observed mixture toxicity.

At present, the TK-TD model has some limitations in predicting the toxicity of metal mixtures with synergistic or antagonistic effects, which is mainly due to the limited understanding of the mechanisms of possible interactive effects during the processes of distribution, transformation, metabolism and toxicity after metals have entered organisms. This was a key conclusion of the Farley et al. (2015) and Farley and Meyer (2015) analyses of the performance of different metal mixture models with a common dataset (4 BLM models and F_{tox}). Only one of the 5 models could predict a Cu-Cd antagonistic dataset, and another one did so by adjusting its BLM parameters specifically to fit that antagonistic dataset.

3.3. Main advantages and disadvantages in predicting toxicity of metal mixtures

These models take into account the dynamic exposure characteristics of the actual environment, and thereby improve toxicity prediction and risk assessment of metal mixtures. Specifically, they are superior in revealing the intoxication process of metal mixtures, describing the accumulation process of metals in organisms over time, and effectively evaluating the ecological effects under complex exposure conditions. It is also a method to effectively extrapolate from experimental conditions to other exposure conditions. In addition, using this model can explain the toxicity mechanism based on experimental data, further expanding the possibility of extrapolating toxicity between metals and between test species. Nevertheless, these implementations are based on model parameter calibration, which requires more intensive sampling for deriving more variable input. What is more, some model limitations lie in predicting metal mixture toxicity with synergistic or antagonistic effects as discussed above.

3.4. Future perspectives

The process-based model is an efficient tool for real-time prediction of metal toxicity, which can mechanistically link the accumulation of metals in organisms over time with toxicity. To improve the predictive capacity of the TK-TD model for metal mixtures, further efforts should focus on investigating the dose-dependent toxicity indicator at the molecular level (i.e., molecular initiating event), and then integrating it into toxicity modeling. Moreover, metal bioavailability is strongly affected by environmental chemical conditions. How to integrate these influences into the current TK-TD model framework so as to more accurately predict and assess the toxicity of metal mixtures in the actual environment will be an important challenge in the future.

4. Omics-based approach

4.1. From genomics to metabolomics: concepts and principles

Conventional ecotoxicological studies mainly focused on the responses of the overall phenotypic level of the organism. In recent years, the use of "omics-based approaches", which can provide information either at the gene, protein or metabolite level, greatly promotes a comprehensive understanding of the molecular mechanisms underlying toxicity. It is not surprising that omics techniques spread to ecotoxicology, which open up new perspectives for investigating the toxicity of toxic substances at the molecular level (Prat & Degli-Esposti, 2019). The "omics" represents "as a whole" genomics, transcriptomics, proteomics, and metabolomics (Figure 5).

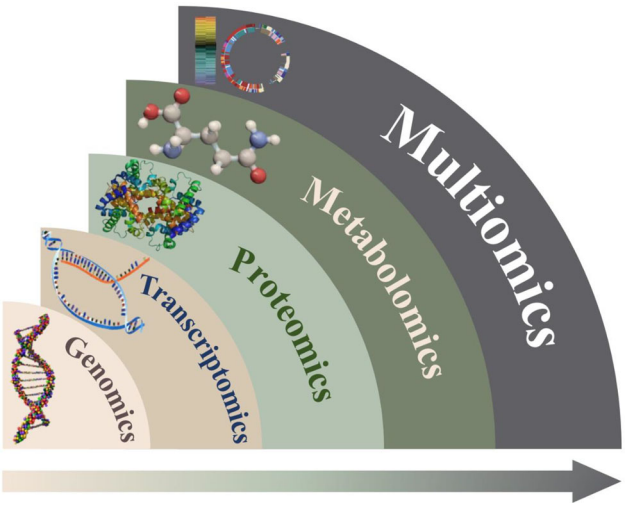

Figure 5. Brief description of the Omics-based approach. The "omics" represents "as a whole" genomics, transcriptomics, proteomics, and metabolomics model.

Genomics study all the nucleotide sequences, including structural genes, regulatory sequences, and noncoding DNA segments, in the chromosomes of an organism and thus identify underlying factors dominating the variability of toxicological responses at the genetic level. This requires an interdisciplinary approach because of the diverse responses involving molecular biology, physiology, toxicology, and so on. Genomics can provide useful information for assessing biological responses following exposure to contaminants, e.g. by the identification of novel biomolecules that may act as biomarkers in environmental monitoring (Adam et al., 2007; González-Fernández et al., 2008; Lindon et al., 2005; Menzel et al., 2009; Montes Nieto et al., 2010; Montes-Nieto et al., 2007; Poynton & Vulpe, 2009; Ruiz-Laguna et al., 2006; Waring et al., 2001).

Genetic responses upon chemical exposure are commonly regulated at the transcriptional level. Transcriptomics can quantify the levels of nearly all the transcriptional profiles to stress conditions. Microarrays are used to measure expression profiles of mRNA, which can help to generate a wide impression of how environmental stressors affect organisms. High-throughput RNA-sequencing (RNA-Seq) technology opens research opportunities for collecting transcriptomic data from any species of interest (Trapp et al., 2016). In addition, quantitative PCR (qPCR) is becoming more important for in-depth gene expression analysis as it allows to quantify a particular fragment in a sample (Altenburger et al., 2012). Recently, transcriptome analysis has become a useful tool to unravel the role of differential expression induced by different gene-related aspects during biological processes (Shi & He, 2014).

The proteome is approximately 10-30 times larger than the transcriptome. Covalent modifications and various interactive effects (e.g., cell-cell, protein-protein and protein-ligand) are responsible for this variability. The proteome is dynamic due to changeable protein functions resulting from such modifications (Efferth & Greten, 2012). Protein expression levels are the product of the process of protein transcription, translation and degradation within cells, including the different stages of maturation and modification within transcripts and proteins. Proteomics can provide additional and supplementary information to transcriptomics through globally analyzing these proteins. This approach also contributes to a broad comprehensive understanding of underlying mechanisms of intoxication by identifying significantly altered proteins within an organism after being exposed to a toxicant. The identified proteins can thus be novel biomarkers in environment biomonitoring (Garcia-Sevillano et al., 2014).

Together, genomics, transcriptomics, and proteomics can provide information on processes at the cellular level, however, in order to further connect genotype to phenotype another layer of information is needed (Fell,

2001). Metabolomics can bridge this gap and provide quantitative information at the intracellular metabolic level which stands for the supreme level of functional components of cellular processes (Fiehn, 2002; Halama, 2014). The metabolites, defined as the metabolome, act as the cell's supplements composed of small and low molecular weight compounds, which are necessary for growth, function and maintenance (Quanbeck et al. 2012). The goal of metabolomics is to systematically identify and quantify these compounds and to report the most relevant information to the phenotype under genetic and/or environmental changes in the biological system (Barupal et al., 2012; Fiehn, 2002; Mashego et al., 2007). Previously, the omics-based approach was often used alone in practical applications. Nowadays, the multiomics methodology has become a popular and revolutionary approach in comparison to single omics, which gathers information from multiple layers and allows to understand better the complex mechanisms of intoxication and defense that act in organisms.

4.2. Application in assessment of metal mixture toxicity

The endpoints in traditional toxicity studies (e.g., survival, reproduction and growth) may have low sensitivity in detecting possible biological effects of exposure to low levels of stressors in the environment. In comparison, omics-based studies on effects of contaminants at low/sub-lethal concentrations have shown high sensitivity (Zhang et al., 2017). This indicates that the risk assessment of toxicants, especially following environmentally relevant exposure scenarios, cannot exclusively depend on traditional target-oriented effects (Martins et al., 2019). In order to apply the omics-based approach to ecotoxicology, it is necessary to relate molecular data obtained from omics-based studies to conventional toxicological endpoints (Vandenbrouck et al., 2010). These associations across different levels of biological organization can provide the basis for models that describe the toxicity of metal mixtures.

Compared with the extensive studies on metal mixture toxicity based on conventional ecotoxicological methods, only few investigations related to the omics-based approach have been reported (Altenburger et al., 2012). These available attempts opened new possibilities to decipher the complicated molecular mechanisms caused by metal mixtures (Table 5). Bae et al. (2002) identified genetic changes in human keratinocytes subject to a quaternary mixture of As, Cr, Cd and Pb using DNA microarray analysis. They suggested that metal mixtures triggered unique gene expression patterns compared to single metal exposures. Mumtaz et al. (2002) found that there is no evidence for synergistic activation of gene expression by a ternary mixture of Cd, Cr and Pb in a commercially developed assay system

Table 5. Overview of the omics-based approach fits to different metal mixture toxicity data.

Metal mixtures	Test species	Omics used	Notes	References
As-Cr-Cd-Pb	human keratinocyte cell line (RHEK-1)	DNA microarray	Metal mixtures triggered unique gene expression patterns compared to single metal exposures.	(Bae et al., 2002)
Cd-Cr-Pb	HeLa cells	genomics	No evidence was found for synergistic activation of gene expression by metal mixture.	(Mumtaz et al., 2002)
Ni-Cd, Ni-Pb	daphnid, *Daphnia magna*	DNA microarray	Metal mixtures affected pathways, suggesting interactive molecular responses rather than simply additive effects of the individual metals.	(Vandenbrouck et al., 2009)
Cu-Cd, Pb-Cd	alga, *Chlamydomonas reinhardtii*	transcriptomics	Synergism and antagonism depended on gene expression levels.	(Hutchins et al., 2010)
Cd-Cu	mussel, *Perna viridis*	metabolomics	Cu dominantly induced the metabolic disturbances.	(Wu & Wang, 2010)
Cd-Pb	mussel, *Mytilus edulis*	transcriptomics	The unfolded protein response (UPR) was determined as early indicator of stress.	(Poynton et al., 2014)
Cu-Cd, Cu-Pb, Cd-Pb	algae, *Chlorella* sp.	metabolomics	Metal mixtures triggered synergistic effects on photosynthesis inhibition, oxidative stress and membrane degradation.	(Zhang et al., 2015)
Cu-Ni	daphnid, *Pulex-pulicaria*	metabolomics	The reduced fecundity could be explained based on metabolic responses determined in juvenile daphnids exposed to acutely (48 h) toxic media.	(Taylor et al., 2016)
Al-In	daphnid, *Daphnia magna*	transcriptomics	Al and In may alter the expression of genes involved in energy metabolism processes to explain reduced growth and reproduction.	(Brun et al., 2019)
Cd-Pb	plant, *Brassica oleracea* and *Trifolium repens*	genomics	The interactive effects between Cd and Pb were concentration- and time-dependent.	(Lanier et al., 2019)
Se-As	rice, *Oryza sativa*	transcriptomics and proteomics	The responsive pathways, genes and proteins of Se in alleviating As toxicity in rice plants were determined.	(Chauhan et al., 2020)
Pb-As, Pb-MeHg, As-MeHg	HT-22 cells	proteomics	The protein expressions were significantly different between single metals and metal mixtures exposure.	(Karri et al., 2020)

CAT-Tox (L). Duarte et al. (2008) found that the effects of Cu-Zn mixtures on microbial decomposition of leaf litter were mainly additive, because observed responses were similar to those anticipated as the sum of individual metal effects. Given that microbes play an irreplaceable role in maintaining human health and the material cycle of the earth's ecosystem, it is of great significance to apply omics approaches to investigate the effects of metal mixtures to microbes. Vandenbrouck et al. (2009) investigated the toxicity of binary metal mixtures (Ni-Cd, Ni-Pb) to *Daphnia magna*. Their results showed additionally affected pathways following exposure to the mixtures, suggesting interactive molecular responses rather than simply additive effects of the individual metals. Hutchins et al. (2010) showed that the addition of Cu and Pb reduced Cd biouptake in *Chlamydomonas reinhardtii*, while the upregulation of the mRNA levels of 6 genes indicated no Cd specificity. The authors revealed synergism and antagonism depending on gene expression levels. Wu and Wang (2010) studied the toxicological effects on green mussels *Perna viridis* exposed to a binary mixture of Cd and Cu, and revealed that Cu dominated metabolic profile changes. Poynton et al. (2014) conducted molecular toxicology of metal bioaccumulation in the blue mussel, *Mytilus edulis* exposed to Cd + Pb mixtures through transcriptomic analysis. They revealed that the unfolded protein response (UPR) served as early indicator of stress. Zhang et al. (2015) investigated the effects of multimetal systems (Cu, Cd, Pb) on freshwater microalgae (*Chlorella* sp.) using a combination of metallomics and nuclear magnetic resonance spectroscopy (NMR)-based metabolomics. They confirmed synergistic effects of Cu and Cd measured as photosynthesis inhibition, oxidative stress and membrane degradation. Taylor et al. (2016) developed a statistical model to predict chronic Cu and Ni reproductive toxicity to *Daphnia pulex-pulicaria* integrating data from a standard chronic, partial life-cycle toxicity test and metabolomics. They also found that reduced fecundity could be explained based on metabolic responses determined in juvenile daphnids exposed acutely (48 h) to the metal mixtures.

More recently, Brun et al. (2019) investigated the combined toxicity of Al and In to *Daphnia magna* at both the phenotypic and the toxicogenomic level. They found a consistent synergistic effect at both levels in Al and In mixtures. They also revealed that these elements may alter the expression of genes involved in energy metabolism processes to explain reduced growth and reproduction. Lanier et al. (2019) conducted acute and long-term (3-, 10- and 56-day exposure) toxicity tests to examine the single and mixed toxicity of Cd and Pb in two plant species (*Brassica oleracea* and *Trifolium repens*). The results showed concentration- and time-dependent interactive effects between Cd and Pb according to the DNA damage

analysis. Chauhan et al. (2020) explored molecular mechanisms of Se ameliorated As induced toxicity in rice plants (*Oryza sativa*) using the integrated omics (transcriptomic and proteomic) approach. The authors identified the responsive pathways, genes and proteins of Se in alleviating As toxicity in rice plants. Karri et al. (2020) investigated the role of binary metal mixtures (Pb, As, MeHg) in neurodegenerative diseases using proteomics analysis. They found that the protein expressions were significantly different between single metals and metal mixture exposures. Hence, omics-based approaches are of great importance for interpreting possible toxicological mechanisms and might identify the pathways by which metal mixtures exert toxicity.

4.3. Main advantages and disadvantages in predicting toxicity of metal mixtures

Omics-based approaches are potential tools for identifying novel molecular mechanisms of metal mixture toxicity in a variety of organisms that would be hard to elucidate through other traditional techniques. Using a single omics technology will only obtain one aspect of toxicity mechanisms. Multi-omics approaches promise to fill the gap and provide a multilevel insight in the mechanisms underlying toxicity. However, these technics are demanding and can be expensive, which make them difficult to promote on a large scale. Other issues that are significant challenges to be addressed include reasonable experimental design, effective data analysis, and integration with other approaches.

4.4. Future perspectives

As the omics-based approaches continue to move forward, a major challenge in making use of these approaches lies, actually, in finding ways to convert the data of multivariate omics to a legible endpoint, to allow estimating the dose-effect relationships, and to quantify metal mixture effects. In addition, the work of discovering mysterious relationship between the phenotypic and molecular interactive effects for complex mixtures by developing adverse outcome pathways will continue to be required. Consequently, efforts need to be continuously implemented to predict the toxicity of metal mixtures and to understand their underlying toxicity mechanisms on the basis of genotypes and phenotypes. It is expected that after further development these techniques can provide a wealth of information not gainable in any other way for investigating metal mixture toxicity.

5. Guidance for selection and assessment approaches

As shown in Tables 1–5, several predictive models have been developed and applied to investigate the mixture toxicity of various metal combinations to certain organisms. It is not surprising that the outcomes of different approaches vary a lot across different test species, different combinations of metals, and different exposure mediums. Hence, it is hard to conclude on an optimal approach to evaluate the joint toxicity for any metal combinations since the predictive capacity of a certain model varies for specific cases. Nevertheless, the relative strengths and limitations of different bioavailability-based methods can provide an initial basis for the selection of models for predicting the toxicity of metal mixtures.

6. Concluding remarks and future prospects

In the environment, metal mixture toxicity is hard to predict due to overlooking potential interactive effects. Much of the focus has virtually been on single metals generating a drought of information on mixture toxicity. For metal mixtures, the use of relatively simple conceptual methods (CA and IA) without considering interactive effects is sometimes unavoidable and appropriate. In most cases, metal toxicity can be affected by the presence of another metal, leading to deviations of the observed effects from additivity in a less-than-additive or more-than-additive manner, which indicate the occurrence of interactive effects at various levels. When the non-interactive assumption of metal components in mixtures is invalid, different approaches are therefore required to account for the interactive effects of mixture components in predicting the toxicity of metal mixtures. Bioavailability-based methods with diverse mechanistic perspectives, such as BLM, ETM, WHAM-F_{tox}, and the TK-TD and omics-based approaches, can offer comprehensive information for better understanding of the underlying mechanisms of joint toxicity.

Until now, the extended BLM is still viewed as rather suitable for mechanistic modeling of metal mixtures based on its theoretical basis of site-specific competition. The electrostatic theory acknowledges the importance of ion-organism interactive effects induced by the electrical potential at the plasma membrane surface of organisms. This approach provides an alternative to the BLM in the assessment of metal bioavailability and toxicity in mixture scenarios from the perspective of electrostatic effects, but so far it has only been applied to plants. Assuming that interactive effects of metal components and protons in mixtures occur at reversible binding sites, the WHAM-F_{tox} approach related to bioavailability can also serve as a simple and alternative method in describing metal mixture toxicity because it easily enables predicting multimetal toxicity with over two components. TK-

TD models provide an elaborate framework for predicting bioaccumulation and toxicity of metals in mixtures. These models are process-based, modular, quantitative, and dynamic. These advantages enable TK-TD models to investigate the potential mechanisms of metal mixture toxicity at different levels, to interpret toxicity data more mechanistically, to provide more environmentally relevant toxicity metrics, such as no effect concentrations, and to extrapolate among different exposure scenarios and even different biological species. According to conventional toxicity endpoints, these methodologies can provide useful information contributing to elucidate the underlying mechanisms of metal mixture toxicity. Mechanistic pathways of metals inside organisms, however, are poorly known. Omics-based approaches would be potentially supplementary to traditional toxicity tests, and provide molecular level information connecting genotype to phenotype for achieving an elaborate panorama of the relevant mechanisms of metal mixture toxicity. In the context of environmental risk assessment of metal mixtures, this review timely summarizes the existing predictive tools and their underlying mechanisms and highlights the importance of integrating mixture interactive effects and bioavailability in assessing the toxicity of metal mixtures.

Most of the existing ecotoxicity tests and relevant studies only focused on the response of partial developmental stages in the full life cycle of target organisms based on limited ecotoxicological endpoints. In fact, organisms may be exposed to metals at different developmental stages throughout their life cycle. Thus, toxicity data only based on exposure of a specific growth stage of organisms may lack environmental relevance. It is recommended to investigate interactive effects during the full life cycle, which might be beneficial to provide more information to interpret metal mixture toxicity. In the long run, future studies should continue investigating the mechanisms of mixture interactive effects and identify the principles of combined toxicity for developing predictive models. Potential topics include, for example, considering the internal distribution and detoxification mechanisms (i.e., toxicological bioavailability) of one metal in the presence of other metals, and across different species of organisms at diverse doses during dynamic exposures and full life cycles. Global information at the molecular level generated by omics-based approaches is expected to be integrated based on mathematical and statistical methodologies to improve existing knowledge and create new discoveries for addressing potential risks induced by metal mixtures.

Declaration of interest statement

There is no competing interest to declare.

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