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Metal mixture toxicity to aquatic biota in laboratory experiments: Application of the WHAM-F_{TOX} model

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ABSTRACT

The WHAM-F_{TOX} model describes the combined toxic effects of protons and metal cations towards aquatic organisms through the toxicity function (F_{TOX}), a linear combination of the products of organism-bound cation and a toxic potency coefficient (α_i) for each cation. Organism-bound, metabolically-active, cation is quantified by the proxy variable, amount bound by humic acid (HA), as predicted by the WHAM chemical speciation model. We compared published measured accumulations of metals by living organisms (bacteria, algae, invertebrates) in different solutions, with WHAM predictions of metal binding to humic acid in the same solutions. After adjustment for differences in binding site density, the predictions were in reasonable line with observations (for logarithmic variables, $r^2 = 0.89$, root mean squared deviation = 0.44), supporting the use of HA binding as a proxy. Calculated loadings of H^+, Al, Cu, Zn, Cd, Pb and UO_2 were used to fit observed toxic effects in 11 published mixture toxicity experiments involving bacteria, macrophytes, invertebrates and fish. Overall, WHAM-F_{TOX} gave slightly better fits than a conventional additive model based on solution concentrations. From the derived values of α_i, the toxicity of bound cations can tentatively be ranked in the order: $H < Al < (Zn–Cu–Pb–UO_2) < Cd$. The WHAM-$F_{TOX}$ analysis indicates much narrower ranges of differences amongst individual organisms in metal toxicity tests than was previously thought. The model potentially provides a means to encapsulate knowledge contained within laboratory data, thereby permitting its application to field situations.

1. Introduction

The biotic ligand model (BLM, Paquin et al., 2002) was developed to explain how water chemistry (pH, DOC, hardness etc.) affects toxicity, initially for single metals. The essential idea is to replace metal concentration in solution as the expression of toxic exposure, by the occupancy of a key (biotic) ligand, the reactions of which are described with conventional coordination chemistry. Account can thus be taken of the ever-present competition reactions, in which toxic and non-toxic cations, including H^+, compete for binding to ligands, not only the biotic ligand but also those present in solution, in particular dissolved organic matter. The aim of the BLM was to make risk assessment more scientific, compared to the use of a single standard concentration, or perhaps hardness-dependent values. This aim has largely been achieved, changing how we think about metal toxicity in aquatic and terrestrial ecosystems. Furthermore, there have been several efforts to use the BLM to account for the toxic effects of metal mixtures (Playle, 2004; Hatano & Shoji, 2008; Kamo & Nagai, 2008; Jho et al., 2011), each based on the assumption that the different toxic metals share the same biotic ligand. Hatano & Shoji (2008) fitted the model to data for the toxicity of Cu and Cd to *Lemna paucicostata*, at different pH values, and obtained far better agreement with observations than could be achieved with a conventional model based on LC_{50} toxic units, and ignoring pH variations.

The WHAM-F_{TOX} model (Stockdale et al., 2010) provides a different way of describing metal toxicity, while retaining the idea that exposure depends on the interactions of metals and protons with the organism. Instead of postulating a specific biotic ligand through which metal toxicity is mediated, WHAM-F_{TOX} expresses exposure of the organism to toxic metals by the overall, non-specific, accumulation of cations at the reversible binding sites present within the organism or on its surface. Such sites exist due to the presence of weak-acid groups in different biomolecules (e.g. proteins, polysaccharides, lipids, nucleic acids, fatty acids), and their occupancy depends upon the competitive interactions of toxic and non-toxic metals and protons, assuming them to be in equilibrium with the surrounding solution. The binding ligands could, in principle, include one or more specific biotic ligands but the majority of them will not be associated directly with the toxic response. The model then assigns a unique, purely empirical, toxicity coefficient to each cation, which describes the extent to which the bound cation is toxic. Total toxicity is then determined by the sum of the products of amounts bound and the toxicity coefficients. The

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chemical interactions and toxic effects are thus formally separated, unlike in the BLM where the equilibrium constants for binding at the biotic ligand reflect not only the chemical strength of binding, but also toxic strength (Playle, 2004).

An advantage of the WHAM-F_{TOX} approach is that the need to fit the model to organism-bound metal data is avoided, by assuming that metal accumulation by living organisms can be estimated with a pre-existing chemical speciation model, i.e. WHAM, using cation binding by humic acid (HA) as a proxy. Evidence to justify this assumption comes from field data (Tipping et al., 2008; Stockdale et al., 2010), although as yet it must be regarded as incomplete. But the idea is worth pursuing in order to avoid an inordinate amount of new experimental work and associated modelling to quantify cation accumulation by living organisms (cf. Borgmann et al., 2008). If cation accumulation can reliably be estimated a priori, then relatively few parameters are needed to fit toxicity data. The use of cation binding to HA, calculated with WHAM, to express metal exposure produced a good description of the toxicity of copper towards duckweed in laboratory experiments (Antunes et al., 2012).

In previous work with WHAM-F_{TOX}, we focused only on field data, firstly using stream macroinvertebrate species richness, at *c.* 400 sites affected by abandoned metal mines and acid deposition, as the toxic response variable for fitting (Stockdale et al., 2010). The same version of the model was used to evaluate acidification recovery (Stockdale et al., 2013). Analysis of stream mesocosm data (Iwasaki et al., 2013) further supports the use of calculated binding to HA as a measure of exposure. However, under circumstances pertaining in the field and in mesocosms, non-chemical factors (e.g. discharge variation, suspended sediment, competition, predation, food web structure) also affect the measured variables, hampering the precise and unequivocal attribution of toxicity. Therefore the principal aim of the present work was to test the ability of the model to fit toxicity data obtained in controlled laboratory experiments. Before addressing toxicity however, we first tested the other aspect of the model, i.e. its appropriateness as a proxy for cation accumulation by living organisms. The longer-term goal of this work is to produce a model that can be parameterised with the abundant laboratory data that describe metal toxicity, in order to make use of the fundamental knowledge to understand and predict toxic effects of metals in the field.

2. Methods

2.1. Modelling chemical speciation with WHAM

In this work we used WHAM (Tipping, 1994) incorporating humic ion-binding model VII (Tipping et al., 2011). Model VII uses a structured formulation of discrete, chemically-plausible, binding sites for protons in humic and fulvic acids (HA, FA), in order to allow the creation of regular arrays of bidentate and tridentate binding sites for metals. Metal aquo ions (Al^{3+}, Cu^{2+}, Cd^{2+} etc.) and their first hydrolysis products ($AlOH^{2+}$, $CuOH^{+}$, $CdOH^{+}$ etc.) compete with each other, and with protons, for binding. The same intrinsic equilibrium constant (K_{MA}) for binding to carboxyl or type A groups is assumed to apply to the aquo ion and its first hydrolysis product. The constant (K_{MB}) for binding to weaker acid groups is related to K_{MA}, and the contributions of rarer "soft" ligand atoms are factored in. The intrinsic equilibrium constants are modified by empirical electrostatic terms that take into account the attractive or repulsive interactions between ions and the charged macromolecule.

The humic ion-binding model is combined with an inorganic speciation model, the species list and constants for which were given by Tipping (1994). The inorganic reactions in this database are restricted to monomeric complexes of metals. The effects of ionic strength on the inorganic reactions are taken into account using the extended Debye–Hückel equation. Temperature effects on reactions between inorganic species are taken into account using published or estimated enthalpy data, but in the absence of experimental information, reactions involving humic substances are assumed to be independent of temperature.

If dissolved organic carbon (DOC) was present in the solutions considered here, we took complexation into account by assuming dissolved organic matter (DOM) to be 50% carbon, with 65% of sites active with respect to cation binding, represented by FA (Tipping et al., 2008). For example, a DOC concentration of 5 mg L^{-1} corresponds to a FA concentration of 6.5 mg L^{-1} for modelling. For waters from the field, we estimated Fe(III) concentrations with the empirical equation of Lofts et al. (2008), suitably modified for humic binding model VII.

We calculated the equilibrium binding of the metals to HA by assuming it to be present at a very low concentration, insufficient to affect the bulk speciation, and finding ν_i values (mol/gHA). To match the values of ν_i to observed accumulations of metal by living organisms, we defined the equivalent HA per gram of organism dry weight, E_{HA} (g g^{-1}). The value of E_{HA} would be 1.0 if the organism's site content per gram were equal to that of HA, but is expected usually to be less than 1.0 because living organisms generally have fewer exposed sites than does HA.

2.2. Fitting toxicity data with WHAM-F_{TOX}

For the toxicity model, it is assumed that each organism possesses binding sites that have the same properties as those of HA, and it is the fractional occupancy of these sites that measures exposure to cations, not the absolute amount of metal per unit weight of organism. Different species exposed to the same solution have the same ν_i values but differ in absolute body burdens (mol g dry weight^{-1}) if their values of E_{HA} differ. Thus, because only relative binding is needed, the model simply uses the calculated ν_i values for HA as the measure of exposure. This means that toxicity parameters for different organisms are directly comparable.

The toxicity function is defined by the equation;

$$F_{TOX} = \Sigma \alpha_i \nu_i \quad (1)$$

in which α_i is the toxicity coefficient of cation *i*. The toxic response (TR), on a scale from zero to unity, depends upon lower and upper thresholds of F_{Tox} according to the following definitions;

$$F_{TOX} \leq F_{TOX,LT} \quad TR = 0 \quad (2)$$

$$F_{TOX,LT} < F_{TOX} < F_{TOX,UT} \quad TR = \frac{F_{TOX} - F_{TOX,LT}}{F_{TOX,UT} - F_{TOX,LT}} \quad (3)$$

$$F_{TOX} \geq F_{TOX,UT} \quad TR = 1 \quad (4)$$

For each data set, the object of the fitting was to minimise the sum of the squared differences between observed and calculated toxic response (luminescence, survival, growth rate or filtration rate). To fit the model, the values of α_i, $F_{TOX,LT}$ and $F_{TOX,UT}$ could in principle be optimised by fitting the model to the available toxicity data. Since the toxicity coefficients are only relative numbers, the value of α_H can be set to the same value in all cases, and unity is chosen for convenience.

2.3. Conventional toxicity model

For comparison with the outputs of WHAM-F_{TOX} modelling, a conventional toxic unit approach was applied to the datasets,