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WHAM-$F_{TOX}\beta$ – An aquatic toxicity model based on intrinsic metal toxic potency and intrinsic species sensitivity

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ABSTRACT

We developed a model that quantifies aquatic cationic toxicity by a combination of the intrinsic toxicities of metals and protons and the intrinsic sensitivities of the test species. It is based on the WHAM-F_{TOX} model, which combines the calculated binding of cations by the organism with toxicity coefficients (α_H, α_M) to estimate the variable F_{TOX}, a measure of toxic effect; the key parameter $\alpha_{M,max}$ (applying at infinite time) depends upon both the metal and the test species. In our new model, WHAM-$F_{TOX}\beta$, values of $\alpha_{M,max}$ are given by the product $\alpha_M^* \times \beta$, where α_M^* has a single value for each metal, and β a single value for each species. To parameterise WHAM-$F_{TOX}\beta$, we assembled a set of 2182 estimates of $\alpha_{M,max}$ obtained by applying the basic model to laboratory toxicity data for 76 different test species, covering 15 different metals, and including results for metal mixtures. Then we fitted the $\log_{10} \alpha_{M,max}$ values with α_M^* and β values (a total of 91 parameters). The resulting model accounted for 72% of the variance in $\log_{10} \alpha_{M,max}$. The values of α_M^* increased markedly as the chemical character of the metal changed from hard (average $\alpha_M^* = 4.4$) to intermediate (average $\alpha_M^* = 25$) to soft (average $\alpha_M^* = 560$). The values of $\log_{10} \beta$ were normally distributed, with a 5-95 percentile range of -0.73 to +0.56, corresponding to β values of 0.18 to 3.62. The WHAM-$F_{TOX}\beta$ model entails the assumption that test species exhibit common relative sensitivity, i.e. the ratio $\alpha_{M,max} / \alpha_M^*$ is constant across all metals. This was tested with data from studies in which the toxic responses of a single organism towards two or more metals had been measured (179 examples for the most-tested metals Ni, Cu, Zn, Ag, Cd, Pb), and statistically-significant ($p < 0.003$) results were obtained.

Key words: Chemical speciation; Metals; Species sensitivity; Toxicity; WHAM; WHAM-F_{TOX}

1. Introduction

A major goal of aquatic toxicity research is to use information gained from laboratory studies to predict toxicity effects in the field. For metals, this requires the toxicity data to be interpreted in terms of bioavailability (water chemistry effects), and account to be taken of the toxic effects of metal mixtures. Then, to predict ecosystem-scale effects, i.e. population responses, the sensitivities of different species to metal toxicity need to be quantified. Here we report the development, parameterisation and testing of a model that combines these features.

The WHAM-F_{TOX} model (Stockdale et al., 2010; Tipping and Lofts, 2013, 2015; Tipping et al., 2019) assumes that cation-binding sites possessed by a biological organism (a) are in chemical equilibrium with the surrounding solution, and (b) can be represented by the binding sites of isolated humic acid (HA). This permits cation accumulation by the organism to be estimated by applying the WHAM chemical speciation model (Tipping et al., 2011; UKCEH 2022), circumventing the need to make numerous new measurements of, for example, metal body burdens, with associated modelling. The combined toxic effect of the bound cations is quantified by the variable F_{TOX}, which is a summation of the products of the occupancy of binding sites by each individual cation and the toxic potency of that cation, denoted by α_H or α_M. The higher is α_M, the more toxic is the metal. In a meta-analysis of published data from multiple sources (2037 individual EC_{50} values referring to single metal-species pairs, taken from 70 different studies, and covering 24 metals and 52 test species), Tipping et al. (2019) found values of α_M by fitting the EC_{50} values, and converted them to $\alpha_{M,max}$ (the value at infinite time) using a generalised time-dependence equation. The derived values of $\alpha_{M,max}$ varied systematically, being strongly correlated with their hardness-softness designations (Pearson, 1963). Therefore, there is evidence of a pattern in the toxic effects of different metals, once solution speciation has been corrected for.

Tipping et al. (2021) argued that for a predictive model to successfully describe field data, biological species would need to exhibit "common relative sensitivity", which means that species differ in a consistent way in their sensitivities to different metals. For example, if species A is twice as sensitive as species B, then a given toxic effect, e.g. 50% mortality, will require only half the exposure to any metal. This concept is essentially the same as that of the "intrinsic sensitivity" of Rubach et al. (2011), who argued that the response of a given species to different toxicants (e.g. metals) is mediated through common (intrinsic) toxicokinetic and toxicodynamic traits. Evidence that this operates for metals comes from the study of Malaj et al. (2012), who analysed the toxic effects of a number of metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn)

towards a range of invertebrate species, using literature data. They employed the "relative sensitivity" variable *S*, introduced by Von der Ohe and Liess (2004), and defined as the logarithm of the ratio of LC_{50} of a metal for *Daphnia magna* to the LC_{50} of the same metal to the species in question. Significant correlations between the *S* values for pairs of metals were found, suggesting that intrinsic sensitivity to different metals is consistent across invertebrate species. Fettweis et al. (2021) measured toxic effects (reductions in growth rate) of three metals (Ni, Cu, Zn) towards 8 different freshwater algal species, under standardised laboratory conditions, and found that metal sensitivities were positively correlated among the species in all three binary combinations (Ni-Cu, Ni-Zn and Cu-Zn).

However, in their meta-analysis, Tipping et al. (2019) did not find convincing evidence of common relative (intrinsic) sensitivity towards different metals among different species, covering three major taxa (invertebrates, plants, vertebrates). The lack of evidence might be explained by modelling shortcomings, in terms of solution speciation, the HA surrogate assumption used in WHAM-F_{TOX}, and the basic model structure. Simple experimental error may also have contributed. Moreover, the toxicity data came from a variety of laboratories, so that the test organisms of the same species could have differed with respect to health and diet (Cowgill, 1987), genotype (Baird et al., 1991) or age (Traudt et al, 2017). The Malaj et al. (2012) study, referred to above, reduced such variability by taking averages from large numbers of observations, while the Fettweis et al. (2021) eliminated the problems by working with the same test strains in carefully-reproduced experiments. Finally, the Tipping et al. (2019) data set may have been too small, unable to provide the necessary statistical power. To attempt to overcome these deficiencies, in the present study we added more toxicity data, largely from recent studies in which mixture effects had been explored. Furthermore, we confined our common relative (intrinsic) sensitivity testing to results that referred to at least two metals, obtained in a single study with the same test species strain. This was expected to eliminate differences between results for different strains of the same organism, and often, since constant water compositions were generally used in individual studies, also to reduce the modelling uncertainties.

To perform the present analysis, we introduce the WHAM-$F_{TOX}\beta$ model in which there is a formal distinction between metal toxic properties and species sensitivity. We tested the hypothesis that there exist two independent sets of parameters, a set of α_M* values that define the toxic potencies of different metals, and a set of β values that define the sensitivities of biological species. The successful fitting of data with WHAM-$F_{TOX}\beta$ would be a step towards

113 the use of the large amount of available laboratory data in the prediction of toxic metal effects
114 in the field.

2. Methods

2.1. Speciation calculations

We used WHAM7 (Tipping et al., 2011; UKCEH, 2022) to calculate solution chemical speciation, taking into account the competitive complexation of major and trace metals with inorganic ligands and dissolved organic matter. As in previous work (Tipping et al., 2008; Stockdale et al., 2010) we attributed dissolved organic matter to fulvic acid (FA), with the standard conversion [FA] (g L^{-1}) = 1.3 [DOC] (g L^{-1}), where square brackets indicate concentrations and DOC is dissolved organic carbon. The key WHAM7 variables characterising the exposure of organisms to cations are $v_{HA,H}$ and $v_{HA,M}$ (mol gHA^{-1}), the amounts of protons and metals bound to humic acid (HA) in equilibrium with the toxicity test solutions. On the assumption that the measured water chemistries represent dissolved concentrations, the proton and metal contents of the organisms themselves were considered negligible, and therefore in order to compute $v_{HA,H}$ and $v_{HA,M}$ we included HA in the calculation inputs at a concentration (10^{-9} g L^{-1}), sufficiently low that the solution speciation would be unaffected by its presence.

2.2. The basic WHAM-F_{TOX} model

Fig. 1 shows a schematic of the calculation procedures, for both the basic model (this Section) and WHAM-$F_{TOX}\beta$ (Section 2.3).

The basic WHAM-F_{TOX} model (Tipping and Lofts, 2013, 2015), recently slightly modified (Tipping et al., 2019), is based on the assumptions that (a) the toxic effects of protons and metal cations are additively related to their occupancies of binding sites possessed by biological organisms, and (b) those binding sites can be represented by the binding sites of humic acid (HA). The dimensionless variables θ_H (for protons) and θ_M (for each metal) are obtained by dividing the $v_{HA,H}$ and $v_{HA,M}$ values from WHAM7 by the HA content of proton-dissociating groups (5.1×10^{-3} mol g^{-1}). See Supplementary Information for further explanation. Evidence that this approach provides reasonable estimates of observed metal body burdens in various biological species has been presented (Tipping et al., 2008; Stockdale et al., 2010; Tipping and Lofts, 2013). It should be noted that the same values of θ_H and θ_M are assumed to apply to any test species exposed to a given solution. However, this is not to say that every species will have the same metal body burden, since that also depends upon the absolute numbers of binding

sites for metals; our assumption is that the fractional occupancies of sites (i.e. θ_H and θ_M) are the same for each species.

In the basic model, the key toxicity variable is F_{TOX}, defined by the equation

$$F_{TOX} = \alpha_H \theta_H + \Sigma \alpha_M \theta_M \quad (1)$$

where α_H and α_M are toxicity coefficients (dimensionless) for protons and metals, and the summation is over all the toxic metals that are present. The equation permits the toxic effects of mixtures of protons and metals to be simulated, taking account of their competitive binding at the HA sites assumed to be possessed by the organism. It includes the assumption that the toxic effects are additive, when exposure is expressed in terms of the amounts of cations accumulated at the organism's binding sites (Stockdale et al., 2010; Tipping and Lofts, 2013, 2015).

The value of α_H is fixed a reference value of 1.00, and is time-independent. Values of α_M depend upon the exposure time employed in a toxicity experiment. In previous work (Tipping et al., 2019), we derived the following relationship to relate α_M to the value at infinite time ($\alpha_{M,max}$) by the equation

$$\alpha_M = \alpha_{M,max}\, kt / 1 + kt \quad (2)$$

where k is a constant (0.77 d^{-1}), and t is the time of exposure (d). Thus, as the exposure time increases, α_M rises towards the maximum value, which means that the value of θ_M required to yield a given F_{TOX} (equation 1) decreases, and the metal effectively becomes more toxic. As a simplifying assumption, the same value of k is assumed to apply to all organisms.

The toxic response (TR) depends upon lower and upper thresholds ($F_{TOX,LT}$ and $F_{TOX,UT}$) of F_{TOX}, between which TR increases linearly from zero to unity. Thus

$$F_{TOX} \le F_{TOX,LT} \qquad TR = 0 \quad (3)$$

$$F_{TOX,LT} < F_{TOX} < F_{TOX,UT} \qquad TR = (F_{TOX} - F_{TOX,LT}) / (F_{TOX,UT} - F_{TOX,LT}) \quad (4)$$

$$F_{TOX} \ge F_{TOX,UT} \qquad TR = 1 \quad (5)$$

In previous work (Lofts and Tipping, 2015; Tipping et al., 2019), in order to avoid over-fitting, the average of $F_{TOX,LT}$ and $F_{TOX,UT}$ (the value of F_{TOX} at which there is a 50% toxicity effect) was fixed at a single value, referred to as $F_{TOX,50}$.

2.3. Extension to WHAM-$F_{TOX}\beta$

In the basic WHAM-F_{TOX} model, the fitted parameter $\alpha_{M,max}$ depends on both the metal and the test species. In WHAM-$F_{TOX}\beta$ the contributions of the metal and the test species are formally separated, according to the equation

$$\alpha_{M,max} = \alpha_M^* \beta \quad (6)$$

Here, α_M^* is the intrinsic toxicity coefficient (applying at infinite time), and there is a single value for each metal. The parameter β is an intrinsic constant characterising the sensitivity of the species towards toxic cations, with a single value for each species. Both α_M^* and β are dimensionless. Equation (6) means that the toxic effect of a metal towards a species is made up of contributions characterising first the metal and second the species. The more potent the metal (higher α_M^*) and the more sensitive the species (higher β), the greater is $\alpha_{M,max}$, and the greater the toxic effect for a given solution composition.

Equation (6) entails the assumption of common relative sensitivity for a given test species. For example, if the values of α_{M1}^*, α_{M2}^* and α_{M3}^* were 1, 20 and 500 respectively, then a species with $\beta = 0.5$ has $\alpha_{M1,max} = 0.5$, $\alpha_{M2,max} = 10$, $\alpha_{M3,max} = 250$, while another species with $\beta = 2.0$ has $\alpha_{M1,max} = 2$, $\alpha_{M2,max} = 40$, $\alpha_{M3,max} = 1000$. The proportions of the $\alpha_{M,max}$ values are the same (1 : 20 : 500) for both species.

It should be noted that the leading term in equation (1), $\alpha_H\theta_H$, is not affected by the value of β, neither does it depend upon exposure time. These assumptions are necessary at present, because data to quantify the relationships are lacking, and they are made in both WHAM-F_{TOX} and WHAM-$F_{TOX}\beta$.

2.4. Data sets and fitting WHAM-F_{TOX}

Literature-derived data used in the present work are summarised below. The toxic responses (mortality, reduced rates of growth, reproduction and filtration), were expressed as the percentage of organisms in a test that were unaffected by the toxic cations (0 - 100 %). Results were accepted if the water compositions in the tests were sufficient to perform speciation calculations with WHAM7; this meant that data on pH, and the concentrations of DOC, major ions and toxic metals were reported, allowing values of θ_H and θ_M to be computed. Source references for the data are given in Supplementary Information. Data fitting was performed with equations (1) – (5) as previously described (Tipping and Lofts, 2013, 2015; Tipping et al., 2019), by minimising the sums of the squared differences between observed and calculated values of the toxic response.

(i) Data from studies in which the toxic effects of protons alone were measured are summarised in Table S2. They refer to 14 different species, 12 of them amphibians, and two invertebrates. The data were combined into a single data set, and this was fitted, maintaining the reference value of 1.00 for α_H, by optimisation of $F_{TOX,LT}$ and $F_{TOX,UT}$ (equations 2 – 4), with equal weight given to the results of each experiment, irrespective of the number of data points. Analysis of these data with the basic WHAM-F_{TOX} model produced a slightly different value of $F_{TOX,50}$, compared to previous work (Tipping et al., 2019); see Section 3.1.

(ii) The data reported by Tipping et al. (2019), comprising 2037 values of EC_{50} from single species-single metal toxicity tests, were reanalysed using the basic WHAM-F_{TOX} model with the revised value of $F_{TOX,50}$. Values of α_M, applying to the duration of the individual experiment, were estimated, using equation (1), then equation (2) was applied to derive $\alpha_{M,max}$.

(iii) Mixture toxicity data assembled by Tipping and Lofts (2013, 2015) were re-analysed using the basic WHAM-F_{TOX} model with the revised (fixed) value of $F_{TOX,50}$. Values of α_M, $F_{TOX,LT}$ and $F_{TOX,UT}$ were estimated first, then $\alpha_{M,max}$ values were obtained with equation (2). See Table S3.

(iv) The same procedures as in (ii) and (iii) were used to analyse new literature data from single- and multi-metal toxicity experiments. See Table S4.

A total of 2182 values of $\alpha_{M,max}$ were derived (Table S5). Of these, 1933 (88.6%) were from measurements of mortality, 164 (7.5%) from measurements of growth impairment, 82 (3.8%) from measurements of the impairment of reproduction, and 3 (0.1%) from measurements of the impairment of filtration rate. Only for the toxic effects of Cu and Zn towards *Daphnia magna* were there sufficient mortality and non-mortality measurements for comparisons to be made. These were done using log $\alpha_{M,max}$ values, to achieve the necessary normal distributions for *t*-tests. In the case of Cu, the average log $\alpha_{M,max}$ for mortality was 1.52 (n = 406), whereas that for non-mortality was significantly ($p < 0.001$) lower at 1.37 (n = 44). In the case of Zn, the corresponding values were 1.17 (n = 35) and 1.24 (n = 21), and the difference was not significant. Given the small difference for Cu and the absence of difference for Zn, we considered it justified to combine results for all types of toxicity effect in our analysis.

2.5. Fitting the WHAM-$F_{TOX}\beta$ model

Values of α_M* (one for each metal, except lanthanides, for which a single overall value was used) and β (one for each species), were estimated from the values of $\alpha_{M,max}$ (Table S5). The

logarithmic version of equation (6) was used to obtain a normal distribution of residuals. The following objective function was minimised;

$$OF = \Sigma (\log \alpha_{M,max} - \log \alpha_{M,max,calc})^2 + w (1 - \beta_{median})^2 \quad (7)$$

Here, $\alpha_{M,max}$ is the value obtained for each data point by applying the basic WHAM-F_{TOX} model (Section 2.4), and $\alpha_{M,max,calc}$ is the value obtained from the parameterised WHAM-F$_{TOX}\beta$ model, i.e. using the values of α_M* and β, depending upon the metal and the test species. The first term on the right-hand side of equation (7) characterises residuals in $\alpha_{M,max}$. The second forces the median β to be close to unity (1.00); this was done because equation (6) implies an infinite number of parameter sets, all fitting the data equally well, since a proportional variation in β can be compensated for by proportionally adjusting the α_M* values. By fixing the median β value, a unique parameter set is obtained. The value of w was set to 500. Fitting was done with the Solver function of Excel^R.

2.6. Testing for common relative sensitivity

Equation (6) means that the ratio $\alpha_{M,max}$ / α_M* (= β) for different metals should be the same for a given species. To test for this, we constructed a data set of paired values of $\alpha_{M,max}$ from results for different metals with the same test species, based on data obtained with the same strain or clone of test species, from experiments in a single laboratory. These pairs are referred to as $\alpha_{M1,max}$ and $\alpha_{M2,max}$. Some were obtained from the earlier Tipping et al. (2019) dataset of EC_{50} values, although only where the study contained more than one estimate of EC_{50}. We added two recently-found values (Table S4). In addition, we used results from experiments with multiple data points. In studies with more than two metals, we took all possible unique pairings. For example if there were three metals (A, B, C), then three separate pairs could be used (A-B, A-C, B-C), if there were four, then there were six pairs, and so on. For each pair, we found two values of β by dividing the $\alpha_{M1,max}$ and $\alpha_{M2,max}$ values by α_{M1}* and α_{M2}* from the full data set fitting (Section 2.5). See Table S6 for the calculated values of β. We restricted the analysis to the six metals (Ni, Cu, Zn, Ag, Cd, Pb) for which there were appreciable numbers of test data. In total, there were 179 paired data, covering 28 different test species.

If the model worked perfectly then the two β values (β_1, β_2) derived from a given pair of α_{M1}* and α_{M2}* would be the same. It would therefore be expected that;

(i) The variance of differences (β_2 - β_1, or log β_2 - log β_1) would be significantly smaller than that of a set of differences generated by random sampling of the individual β values. This was

tested by comparison of the observed differences with a set of 20000 randomly-generated differences. Logarithmic values were used, to make the distributions normal.

(ii) A plot of β_2 against β_1 should have a slope of 1.00 and pass through (1,1), or the logarithmic version would have a slope of 1.00 and pass through (0,0). Again, logarithmic values were used, to make the distributions normal. Since there must be similar errors in the two values, major axis regression (Legendre & Legendre, 2012) was the appropriate way to make the plot, and this was implemented using the lmodel2 package in R (R Core Team, 2017). Because there is no certain way of ordering the pairs of results, i.e. which is β_1 and which β_2, they were selected randomly, and the analysis repeated 2000 times, to obtain representative results.

3. Results

3.1. Fitting or re-fitting toxicity data with the basic WHAM-F_{TOX} model

The combined data set covering the toxic effects of acidity, from experiments without toxic metals, were fitted fairly well with the model (Fig. S1). The mean value of 0.820 for $F_{TOX,50}$ is in good agreement with the value of 0.808 estimated from previous fitting (Tipping et al. 2019), based on data for toxic metals. For the subsequent analyses of metals data in the present work, we adopted the revised value of 0.820 for $F_{TOX,50}$, and maintained α_H at 1.00.

We used the basic WHAM-F_{TOX} model to fit all the cation toxicity data sets with multiple points, to obtain best-fit values of $F_{TOX,LT}$ (and $F_{TOX,UT}$ from $F_{TOX,LT}$ and the fixed value of $F_{TOX,50}$) and the α_M value, or values if the study referred to multiple metals. The basic version of WHAM-F_{TOX} was able to fit, or re-fit, the multiple-point toxicity test data well in the majority of cases (Fig. S2, Tables S3 and S4), all regressions of observed vs. calculated data being significant at $p < 0.001$. Values of α_M from these analyses were converted to $\alpha_{M,max}$ values using equation (2). This yielded a total of 2182 values of $\alpha_{M,max}$ (Table S5).

The number of multi-point data sets that have now been fitted with the basic model is now 61, compared with 15 previously, and therefore we have a fuller set of $F_{TOX,LT}$ and $F_{TOX,UT}$ values. As noted above, these have a forced mean ($F_{TOX,50}$) of 0.820. The mean $F_{TOX,LT}$ value is 0.503, and the mean $F_{TOX,UT}$ is 1.137. The means and standard deviations of the $F_{TOX,LT}$ values for the invertebrates (0.442, 0.217, n = 26) and plants (0.435, 0.237, n =15) are similar, whereas for vertebrates the mean $F_{TOX,LT}$ is 0.633 and the standard deviation is 0.148 (n =20); this reflects the presence of data from a study of 8 fish species in which Al toxicity was followed over time (Poléo et al. 1997), for which sharp transitions were modelled (Fig. S2), leading to relatively high $F_{TOX,LT}$ and low $F_{TOX,UT}$.

3.2. Fitting the WHAM-$F_{TOX}\beta$ model

The objective here was to test the applicability of equation (6), by optimising values of α_M* (one for each metal) and β (one for each species) as described in Section 2.5. The derived values of α_M* are shown in Table 1, and those of β in Table 2. The values of $\alpha_{M,max}$ obtained by fitting the toxicity data with the basic WHAM-F_{TOX} model (Section 3.1, Table S3) are plotted against the values calculated from α_M* and β in Fig. 2.

From equation (6), its is expected that, for a given species, a plot of log $\alpha_{M,max}$ (from the basic model) vs log α_M* should be a straight line with a slope of unity and an intercept of log β, while a plot of log $\alpha_{M,max}$ vs log β for an individual metal should be a straight line with a slope of unity and an intercept of log α_M*. Results for species and metals with many data (Fig. 3), show that these expectations are met. There is considerable scatter in the relationships, but no obvious bias.

The α_M* values of Table 1 are strongly related to the hard-intermediate-soft classification of Pearson (1963), as shown in Fig. 4. The values of α_M* increase markedly as the character of the metal changes from hard (average α_M* = 4.4) to intermediate (average α_M* = 25) to soft (average α_M* = 560). The $\log_{10} \beta$ values are approximately normally distributed (Fig. 5), with a 5-95 percentile range of -0.73 to +0.56, corresponding to β values of 0.18 and 3.62. There are no appreciable variations in averaged β values among the three major taxa (Table 3), in accord with previous findings for $\alpha_{M,max}$ values (Tipping et al. 2019).

3.3. Testing for common relative sensitivity with paired data

The paired values of β for the major six metals (Table S6) were log-normally distributed, as were the differences between the pairs. The variance of the differences was 0.310, significantly ($p < 0.002$) less than the variance of differences generated randomly from the β values, 0.446. This is evidence that common relative sensitivity operates.

Plots of paired log β values against one another, one for each metal, are shown in Fig. S3. The major axis regression slopes are all positive. In four cases (Ni, Cu, Zn, Ag) they are significant ($p < 0.05$), and for Cd the slope is close to significance ($p = 0.054$).

The combined data set of paired values ($n = 179$) was analysed by major axis regression, with 2000 repeated random choices of log β_1 and log β_2. Four examples of the plots are shown in Fig. 6. In all cases, the slope was positive, with $p < 0.003$ (average $p = 0.0012$). In 86 % of cases the 5-95% CL of the slope included 1.00. Thus, the results conform to the expectations of common relative sensitivity (see Section 2.6).

4. Discussion

Variation in the values of $\alpha_{M,max}$ derived from the application of the basic WHAM-F_{TOX} model can be explained significantly with a set of α_M* values and a set of β values (Fig. 2). The resulting parameterised WHAM-$F_{TOX}\beta$ model accounts for patterns in the data, when considered for individual species (Fig. 3). However, there remains considerable data scatter. As mentioned in the Introduction, some scatter must arise from variations in toxicity test results among laboratories. And some will be due to modelling approximations and simplifications; these include the assumption that HA is a surrogate for living material, that the WHAM7 software accurately predicts chemical speciation, and that temporal variation in toxic response is captured by equation (2). It must also be recognised that the data set used for fitting, although quite large, is also biased, towards the toxicity of copper (Table 1) and a few commonly-used test species, especially *Daphnia magna*, *Oncorhynchus mykiss* and *Pimephales promelas* (Table 2). Moreover, the available data for the toxic effects of acidity alone refer mainly to amphibians (Table S2). All these factors limit data interpretation and the drawing of conclusions, but overall the modelling approach makes sense, and appears internally consistent. Therefore this approach has merit, and the results suggest an underlying pattern in metal toxicity towards aquatic organisms, separately dependent upon metals and species.

*4.1. Values of α_M**

The values of α_M* fall into the hard-intermediate-soft (H-I-S) categorisation of Pearson (1963), as shown by the plot in Fig. 4. A similar division was previously published, based on individual $\alpha_{M,max}$ values (Tipping et al., 2019). The idea of using the H-I-S and related systems to classify metals for toxicity was suggested by Nieboer and Richardson (1980), and by Kinraide (2009). However, in these previous studies, comparisons were made in terms of conventional toxicity measures, i.e. solution concentrations of metals. Our approach differs in that it splits the metal interactions into (a) accumulation by the organism, and (b) the toxic effect of bound metal. Our H-I-S pattern for α_M* refers only to the latter. Tipping et al. (2019) interpreted this to mean that, in terms of binding to biological macromolecules, the large, soft metals Ag, Cd and Hg are the most effective in terms of toxicity due to their greater disruptive abilities. Another possibility is that the α_M* values reflect the extent of interaction of metals with protein sulphur centres, especially cysteine, known to be important with respect to protein structure (Wiedemann et al., 2020).

It should be noted that the separation of metal binding and toxic potency means that comparison of α_M* values does not provide a ranking of toxic effect in terms of solution concentrations. Thus, if one metal exhibits strong binding to HA but has a relatively low value of α_M*, it could have a similar toxic concentration to a second metal with relatively weak binding but a large value of α_M*. Actual toxic effects for a given species can only be predicted taking into account both solution speciation and toxic potency.

The list of cationic metals in Table 1 is incomplete, either because we lack parameters for the WHAM7 speciation model, and/or because there are no suitable toxicity test data for analysis. In addition, there is uncertainty about the possible toxic effects of alkaline earth cations. In the WHAM7 speciation model, used as the basis for both WHAM-F_{TOX} and WHAM-$F_{TOX}\beta$, the common cations Mg^{2+} and Ca^{2+} are assumed to bind at the same sites on HA as other metals, but not to exert toxic effects. Thus α_{Mg}* and α_{Ca}* are both equal to zero, and competition by Mg^{2+} and Ca^{2+} towards the binding of other metals at sites in the organism protects against toxicity, consistent with the effects of water hardness (see e.g. Meyer et al., 1999). However, there is evidence that at high enough concentrations these two cations, more especially Mg^{2+}, can exert toxic effects (Biesinger and Christensen, 1972; Mount et al., 1997; Van Dam et al.; 2010). The question then arises as to whether the toxic effects of Mg and Ca are mediated by the same mechanism(s) as those of the metals considered to be toxic in the present analysis (Table 1), or whether they are wholly or mostly due to the interruption of osmotic homeostasis. In the case of the common monovalent ions Na^+ and K^+, their very weak binding to natural organic matter, limited in WHAM7 to electrostatic attraction, means that the apparent toxic effects reported by Biesinger and Christensen (1972) and Mount et al. (1997) would, in the basic WHAM-F_{TOX} and WHAM-$F_{TOX}\beta$ models, have to be attributed to the osmotic effect.

4.2. Values of β

The parameter β quantifies the susceptibility of a species to metal toxicity, and depends on the concept of common relative (or intrinsic) sensitivity of test species. We have found evidence for this (Section 3.3) from analysis of paired data, obtained from studies in which the laboratory conditions and researchers, and the test strains, were likely to be consistent, therefore making comparisons as reliable as possible. As mentioned in the Introduction, previous studies have also provided evidence for common relative sensitivity, by different approaches. Malaj et al. (2012) worked with averaged LC_{50} values for different metal-invertebrate pairs, after rejecting

outliers and normalising for exposure time, temperature and hardness, but not dealing fully with solution speciation. They obtained Pearson correlation coefficients in the range 0.50 to 0.73, in pairwise comparisons of different metals. Fettweis et al. (2021), in a study of the effects of Ni, Cu and Zn on the specific growth rates of 8 algal species under constant standardised conditions, used 10% effect concentrations expressed as free ion activities as a test criterion, and found the log-transformed metal sensitivities to be positively correlated ($p < 0.1$) among the species in all 3 binary combinations (Ni–Cu, Ni–Zn, and Cu–Zn).

The fitted values of β (Table 2) show a 5-95 percentile range of 0.18 to 3.62, a factor of 20-fold. The results refer to a substantial number of different species (76 in all), but caution should be exercised when interpreting the values, because many of them refer to only a few observations. Nonetheless, the results for the 15 species with relatively large numbers of observations follow quite closely the overall pattern (Fig. 5). The descriptive statistics for invertebrates, plants and vertebrates are quite similar (Table 3), so our results do not show any systematic variations in β among these major taxa.

In the WHAM-F_{TOX} and WHAM-$F_{TOX}\beta$ models, the contribution to F_{TOX} of a metal that has accumulated at a metabolically-relevant site is given, from equations (1), (2) and (6) by the product $\alpha_M^* \times (kt / 1 + kt) \times \beta \times \theta_M$. Our starting interpretation of this term is that all organisms have the same values of α_M^*, k and θ_M, so that β is a measure of how susceptible the organism is to the "toxic pressure" quantified by $\alpha_M^* \times (kt / 1 + kt) \times \theta_M$. However, the product might be interpreted in other ways. Firstly β might be a modifier only of α_M^*, and would therefore be a measure of how responsive to bound metal are toxically-sensitive sites possessed by different organisms. Secondly β might be a modifier only of k, measuring how rapidly the metabolically-sensitive sites accumulate metal, faster accumulators being more sensitive. Thirdly, β might quantify differences in the chemistry of accumulation sites, modifying only θ_M. In each case, β distinguishes one species from another, but without defining the actual mechanism(s) by which it does so.

4.3. Theory and toxicity mechanisms

The WHAM-$F_{TOX}\beta$ model belongs in the category referred to by Gong et al. (2020) as a thermodynamic equilibrium toxicity model, since it is based on chemical equilibria. The Biotic Ligand Model (BLM; Pacquin et al., 2002), falls into the same category, but differs in that is based on a single binding site mediating toxic response, as opposed to the collection of

heterogeneous sites that are included in the WHAM model. At the most basic level, these models might be considered simply to be data-fitting devices, comprising collections of equations that permit the efficient combination of mathematical relationships that describe toxic effects.

A second kind of model identified by Gong et al. (2020) the process-based, kinetic, approach, was pioneered by Luoma and Rainbow (2005), who described their "Biodynamic Model" as a mechanistically-based approach to the description of metal bioaccumulation (internal exposure), empirically considering geochemical influences, species differences, and differences among metals. Although simple combinations of the BLM and biodynamic model have been reported (Veltman et al., 2010; Liang et al., 2021) a comprehensive analysis of large data sets, and the ability to deal efficiently with competition effects, are awaited. Through the time-dependence equation (2), both the basic WHAM-F_{TOX} and the WHAM-$F_{TOX}\beta$ models have something in common with the biodynamic model, albeit in a highly simplified way.

Further progress may depend upon identifying the actual biochemical sites of toxic action, including their intracellular and extracellular locations, and then linking the chemical interactions and movements of the metals. Assuming that at least some of the toxic effects occur internally, as assumed by the biodynamic model, this would need to go beyond the original conception of the BLM, in which the key metal-organism interactions took place where the surrounding solution interfaces with the gills of fish and other taxa (Niyogi and Wood, 2004). Multiple sites of action are quite possible.

4.4. Potential field applications

The WHAM-$F_{TOX}\beta$ model is comprehensive in that it quantifies aquatic metal toxicity by taking into account the combined effects of multiple metals, different biological species, the effect of water chemistry on bioavailability, and time dependence. It has been fitted with a considerable data set, although additional laboratory toxicity results, expanding results for metals other than Cu, and covering a wider range of test species, would of course be valuable for model testing and improvement. However, despite such data limitations, the simplifications and approximations used in the modelling, and uncertainties about toxicity mechanisms, discussed above, WHAM-$F_{TOX}\beta$ does appear to have potential for the prediction of field conditions.

We envisage that, in its present form, application of the model would be restricted to estimating the effects of toxic metals on species richness, i.e. the number of difference species that can be identified in a water sample, which is a commonly reported ecological variable. Direct application would entail the assumption that the distribution of β values derived here, and based on results for different laboratory test species, is representative of the β values of field species. This would need to be tested with suitable field data sets, such as those for macroinvertebrates in streams (Stockdale et al., 2010) and zooplankton in lakes (Tipping et al., 2021). Another important issue is the appreciable scatter in the fitting results (Figs. 2 and 3), which will require an error analysis of the parameters, to permit the allocation of uncertainty to the model's predictions. These are the next steps in moving towards making WHAM-$F_{TOX}\beta$ a useful device in the understanding and prediction of the effects of toxic metals in natural waters.

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References

Biesinger, K.E., Christensen G.M. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Bd. Canada 29, 1691-1700.

Cowgill, U.M., 1987. Critical analysis of factors affecting the sensitivity of zooplankton and the reproducibility of toxicity test results. Wat. Res. 21, 1453-1462.

Fettweis, A., Bergen, B., Hansul, S., De Schamphelaere, K., Smolders, E. 2021. Correlated Ni, Cu, and Zn sensitivities of 8 freshwater algal species and consequences for low-level metal mixture effects. Environ. Toxicol. Chem. 40, 2013–2023.

Gong B., Qiu H., Romero-Freire, A., Van Gestel, C.A.M., He, E., 2020. Incorporation of chemical and toxicological availability into metal mixture toxicity modeling: State of the art and future perspectives. Crit. Rev. Environ. Sci. Technol. 52, 1730-1772.

Kinraide, T.B., 2009. Improved scales for metal ion softness and toxicity. Environ. Toxicol. Chem. 28, 525-533.

Legendre, P., Legendre, L. 2012. Numerical ecology. Number 24 in Developments in Environmental Modelling. Elsevier, Amsterdam, 3rd edition.

Liang, W.Q., Xie, M., Tan, Q.G. 2021. Making the Biotic Ligand Model kinetic, easier to develop, and more flexible for deriving water quality criteria. Water Research 188, 116548.

Luoma, S.N., Rainbow, P.S. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39, 1921-1931.

Malaj, E., Grote, M., Schäfer, R.B., Brack, W., von der Ohe, P.C., 2012. Physiological sensitivity of freshwater macroinvertebrates to heavy metals. Environ. Toxicol. Chem. 31, 1754–1764.

Meyer, J.S., Santore, R.C., Bobbitt, J.P., DeBrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., Di Toro, D.M., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. Environ. Sci. Technol. 33, 913–916.

Mount, D.R., Gulley, D.D., Hockett, J.R., Garrison, T.D., Evans, J.M., 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia, Daphnia magna and Pimephales promelas* (fathead minnows) Environ. Toxicol. Chem. 16, 2009–2019.

Nieboer, E., Richardson, D.H.S., 1980. The replacement of the nondescript term "heavy metals" by a biologically and chemically significant classification of metal ions. Environ. Pollut. 1. 3-26.

Niyogi, S., Wood, C.M., 2004. Biotic Ligand Model, a flexible tool for developing site-specific water quality guidelines for metals. Environ. Sci. Technol. 38, 6177-6192.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., et al., 2002. The biotic ligand model: a historical overview. Comp. Biochem. Physiol. C 133, 3–35.

Pearson, R.G., 1963. Hard and soft acids and bases. J. Am. Chem. Soc. 85, 3533-3539.

Poléo, A.B.S., Østbye, K., Øxnevad, S.A., Andersen, R.A., Heibo, E., Vørllestad, L.A., 1997. Toxicity of acid aluminium-rich water to seven freshwater fish species: a comparative laboratory study. Environ. Pollut. 96, 129-139.

R Core Team, 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rubach, M.N., Ashauer, R., Buchwalter, D.B., De Lange, H.J., Hamer, M., Preuss, T.G. et al., 2011. Framework for traits-based assessment in ecotoxicology. Integr. Environ. Assess. Manage. 7, 172–186.

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

Tipping, E., Lofts, S., 2013. Metal mixture toxicity to aquatic biota in laboratory experiments: application of the WHAM-FTOX model. Aquat. Toxicol. 142–143, 114–122.

Tipping, E., Lofts, S., 2015. Testing WHAM-FTOX with laboratory toxicity data for mixtures of metals (Cu, Zn, Cd, Ag, Pb). Environ. Toxicol. Chem. 34, 788–798.

Tipping, E., Vincent, C.D., Lawlor, A.J., Lofts, S., 2008. Metal accumulation by stream bryophytes, related to chemical speciation. Environ. Pollut. 156, 936–943.

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

Tipping, E., Stockdale, A., Lofts, S., 2019. Systematic analysis of freshwater metal toxicity with WHAM-F_{TOX}. Aquat. Toxicol. 212, 128–137.

Tipping, E., Lofts, S., Keller, W. 2021. The use of WHAM-F_{TOX}, parameterized with laboratory data, to simulate zooplankton species richness in acid- and metal-contaminated lakes. Aquat. Toxicol. 231, 105708.

Traudt, E.M., Ranville, J.F., Meyer, J.S., 2017. Effect of age on acute toxicity of cadmium, copper, nickel, and zinc in individual-metal exposures to *Daphnia magna* neonates. Environ. Toxicol. Chem. 36, 113–119.

UK Centre for Ecology and Hydrology, 2022. Windermere Humic Aqueous Model (WHAM7). Available at https://www.ceh.ac.uk/services/windermere-humic-aqueous-model-wham [verified 18 August 2022].

Van Dam, R.A., Hogan, A.C., McCullough, C.D., Houston, M.A., Humphrey, C.L., Harford, A.J., 2010. Aquatic toxicity of magnesium sulfate, and the influence of calcium, in very low ionic concentration water. Environ. Toxicol. Chem. 29, 410–421.

Veltman, K., Huijbregts, M.J., Hendricks, A.J. 2010. Integration of Biotic Ligand Models (BLM) and bioaccumulation kinetics into a mechanistic framework for metal uptake in aquatic organisms. Environ. Sci. Technol. 44, 5022-5028.

Von der Ohe, P.C., Liess, M., 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds Environ. Toxicol.Chem. 23, 150–156.

Wiedemann, C., Kumar, A., Lang, A., Ohlenschläger, O., 2020. Cysteines and disulfide bonds as structure-forming units: insights from different domains of life and the potential for characterization by NMR. Front. Chem. 8:280.

561 Table 1. Values of α_M* obtained by fitting $\alpha_{M,max}$ values from the basic WHAM-F_{TOX} model
562 to equation (6). The designation Ln(III) refers to all trivalent lanthanides combined.

563

Metal	*n*	α_M*
Al(III)	20	1.4
Be(II)	2	2.0
Sc(III)	2	2.8
Mn(II)	11	2.3
Co(II)	9	29.3
Ni(II)	101	19.0
Cu(II)	1574	24.4
Zn(II)	154	12.5
Y(III)	2	1.8
Ag(I)	44	1044.5
Cd(II)	174	464.9
Ln(III)	36	2.2
Hg(II)	5	164.6
Pb(II)	41	41.6
UO_2(II)	7	18.6

564

565 Table 2. Values of β for 76 test species. The larger is β the more sensitive is the species to
566 toxic metals.

Species	*n*	β		Species	*n*	β
Acellus aquaticus	3	0.34		*Lumbriculus variegatus*	11	0.25
Acipenser transmontanus	21	1.32		*Lymnaea stagnalis*	18	1.62
Ambloplites rupestris	1	0.31		*Macrobrachium lanchesteri*	2	0.90
Amerianna cumingi	1	1.14		*Melanoides tuberculata*	2	0.07
Ankistrodesmus falcatus	3	1.53		*Mogurnda mogurnda*	1	1.84
Baetis tricaudatus	1	0.12		*Moinodaphnia macleayi*	1	1.23
Bufo americanus	5	1.99		*Nais elinguis*	2	1.13
Bufo boreas	1	0.53		*Oncorhynchus apache*	1	0.76
Ceratophyllum demersum	12	0.57		*Oncorhynchus clarkii*	15	0.72
Ceriodaphnia dubia	135	1.91		*Oncorhynchus mykiss*	295	1.03
Chironomus dilutus	1	0.08		*Oncorhynchus tshawytscha*	100	0.72
Chironomus javanus	2	0.20		*Perca fluviatilis*	1	1.83
Chlamydomonas reinhardtii	4	0.98		*Phoxinus phoxinus*	1	1.91
Chlorella kesslerii	1	0.24		*Physa gyrina*	2	1.07
Chlorella sp.	1	1.02		*Pimephales promelas*	454	0.96
Chlorella vulgaris	3	0.57		*Poecilia reticulata*	2	0.26
Cottus bairdi	27	1.46		*Poeciliopsis occidentalis*	1	0.51
Danio rerio	22	0.24		*Prosopium williamsoni*	4	2.02
Daphnia ambigua	2	0.45		*Pseudokirchneriella subcapitata*	112	1.39
Daphnia magna	540	1.27		*Ptychocheilus lucius*	2	0.32
Daphnia obtusa	53	1.44		*Pyrgulopsis idahoensis*	6	1.43
Daphnia pulex	54	1.90		*Pyrgulopsis robusta*	2	1.64
Daphnia pulex-pulicaria	2	0.76		*Rana pipiens*	4	1.99
Daphnia pulicaria	34	1.90		*Rasbora sumatrana*	2	0.75
Desmodesmus subspicatus	3	0.60		*Rutilus rutilus*	2	2.40
Dreissena polymorpha	3	0.78		*Salmo salar*	1	2.71
Dugesia tigrina	9	0.20		*Salmo trutta*	1	1.76
Etheostoma flabellare	4	0.32		*Salvelinus alpinus*	1	1.68
Etheostoma lepidum	1	0.42		*Salvelinus confluentus*	51	0.72
Etheostoma nigrum	4	0.28		*Scaphirhynchus platorynchus*	1	0.38
Etheostoma rubrum	1	0.93		*Scenedesmus quadricauda*	3	0.89
Fluminicola sp.	1	1.90		*Stenocypris major*	2	0.80
Fontigens aldrichi	1	1.39		*Synechococcus elongatus*	3	6.64
Hyalella azteca	75	2.32		*Taylorconcha serpenticola*	1	1.75
Hydra viridissima	1	1.23		*Tetraedron minimum*	3	1.12
Lampsilis siliquoidea	28	1.37		*Thymallus thymallus*	1	1.76
Lemna aequinoctialis	3	0.49		*Villosa iris*	4	2.11
Lemna paucicostata	2	0.06		*Xyrauchen texanus*	2	0.45

567

568 Table 3. Log β values summarised for the three major taxa.

	invertebrates	plants	vertebrates
n	31	13	32
mean	-0.09	-0.13	-0.07
SD	0.43	0.47	0.33
median	0.09	-0.05	-0.07

569

Captions to figures

Fig. 1. Schematic of modelling calculations, as described in Section 2.2. The point shown in the lower graph indicates one possible value of F_{TOX}, falling in the range between $F_{TOX,LT}$ and $F_{TOX,UT}$ where a partial toxic effect is predicted.

Fig. 2. Values of $\alpha_{M,max}$ extracted from toxicity data with WHAM-F_{TOX} (Table S5) plotted against values predicted with the WHAM-$F_{TOX}\beta$ model using parameters from Tables 1 and 2. The line is the regression; slope 1.001, intercept -0.0013, r^2 0.723, n 2182.

Fig. 3. Results of model fitting, illustrated with results for many species and many metals. In the upper six panels, values of log $\alpha_{M,max}$ derived with the basic WHAM-F_{TOX} model (Section 3.1) are plotted against values of log α_M*; the lines show the expected relationship, with a slope of 1.00 and an intercept of log β. In the lower six panels, log $\alpha_{M,max}$ values are plotted against log β; the lines show the expected relationship, with a slope of 1.00 and an intercept of log α_M*.

Fig. 4. Values of log α_M* plotted according to the hard-intermediate-soft categories for metals of Pearson (1963).

Fig. 5. Distribution of log β values (n = 76), and the log-normal curve obtained from the mean (-0.09) and standard deviation (0.393). Values for the 15 species with β values obtained from 20 or more observations are highlighted in black. The larger is β the more sensitive is the species to toxic metals.

Fig. 6. Four examples of plots of paired derived log β values (n = 179). The choices of β_A and β_B were made randomly from β_1 and β_2 (see Table S6). Fitted major axis slopes are shown; (a) 0.66, (b) 1.03, (c) 0.79, (d) 0.97. All slopes are significant at p = 0.002.

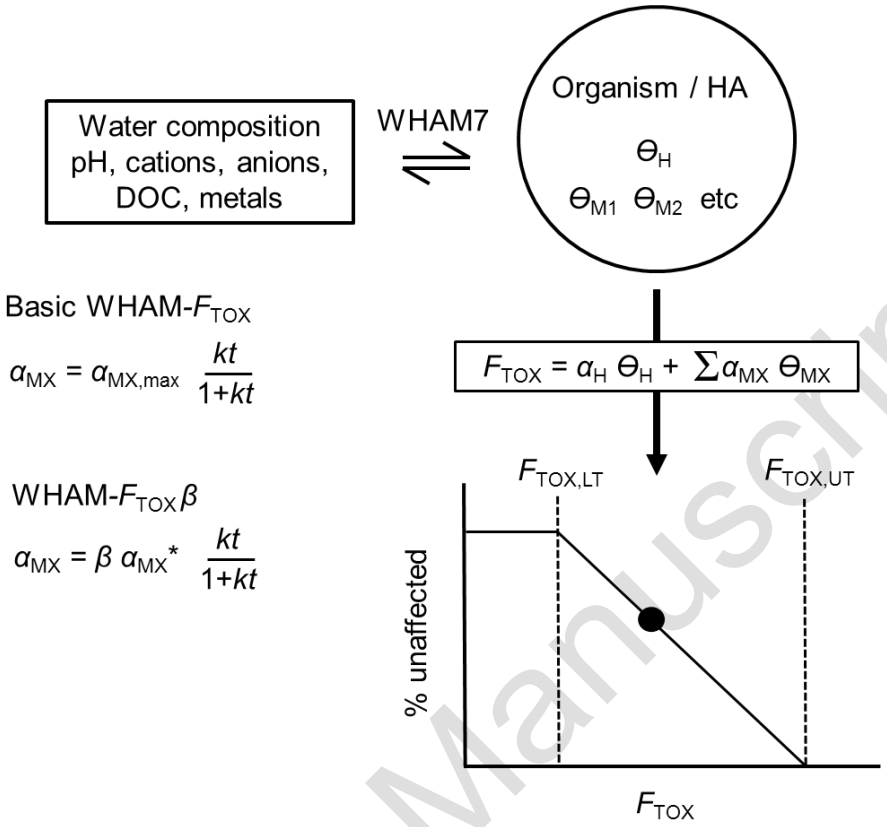


Fig. 1.

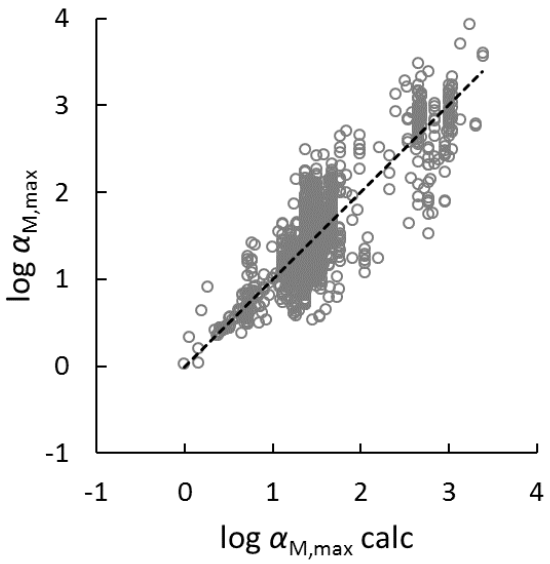
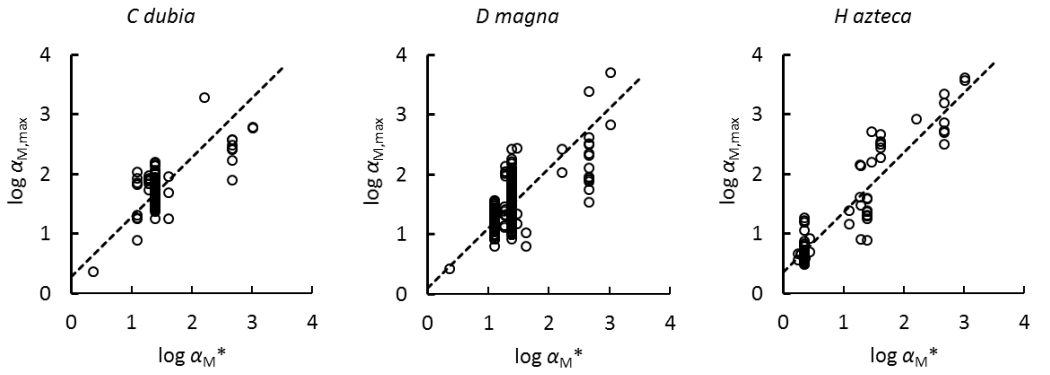


Fig. 2.



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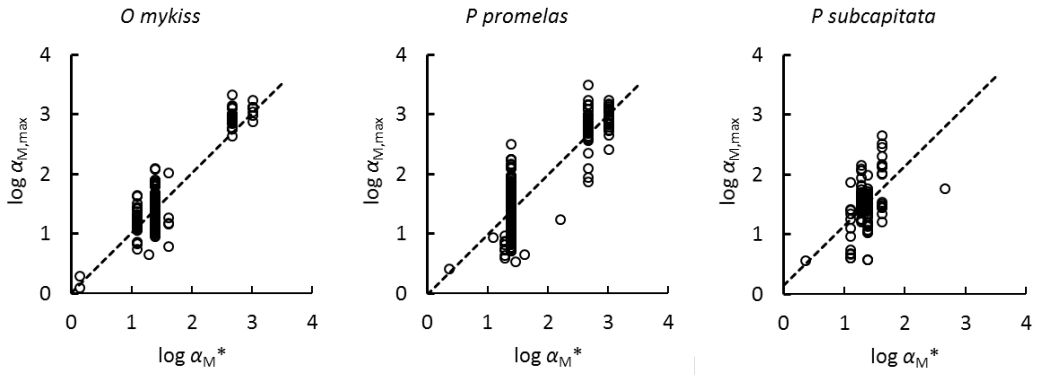

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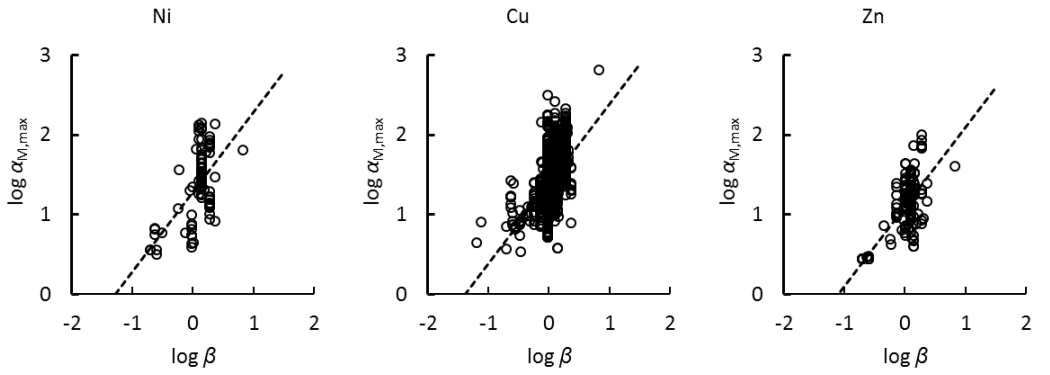

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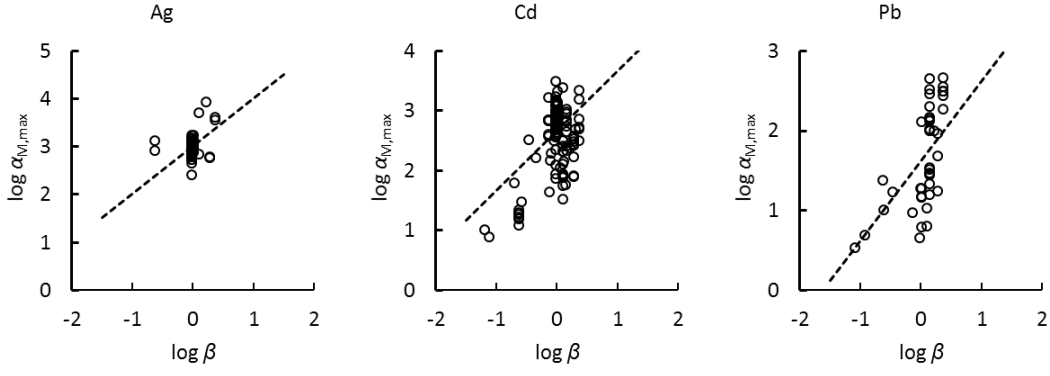

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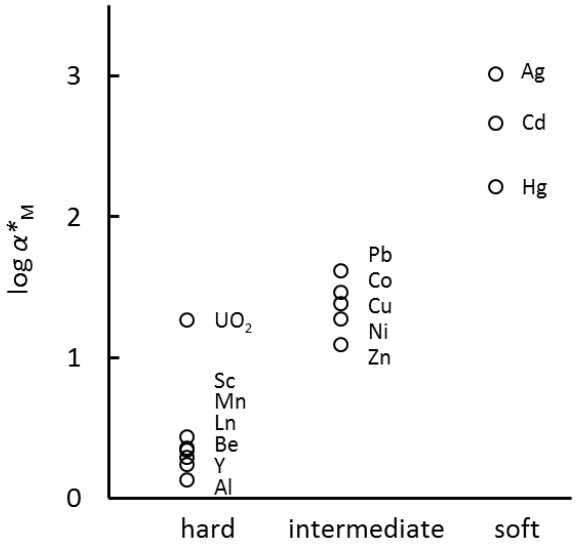
605 Fig. 3.



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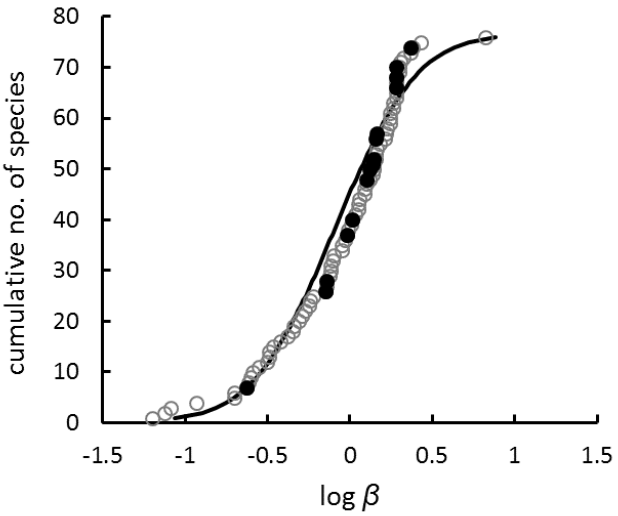

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614 Fig. 5.

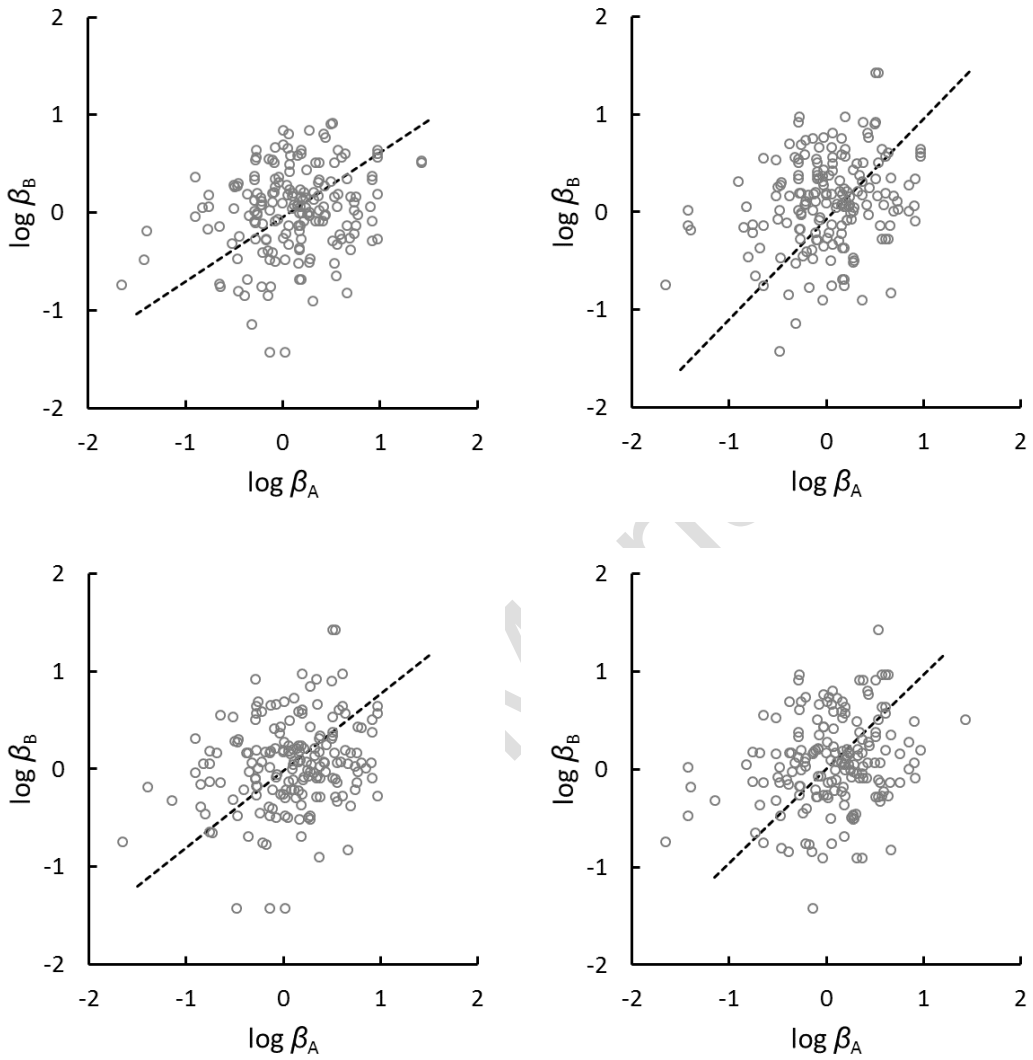

Fig. 6.