Centre for Ecology & Hydrology

NATURAL ENVIRONMENT RESEARCH COUNCIL

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Modelling multiple metal toxic effects in the field – evaluation of the Toxicity Binding Model (TBM)[*]

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[*] During the course of the project it was decided that a better name for the model is WHAM-F_{TOX}

Summary

- Understanding metal and proton toxicity under field conditions requires consideration of the complex nature of chemicals in mixtures. Here, we demonstrate a novel method for relating the stream and river water concentrations of cationic species to a field ecological variable. The model WHAM-F_{TOX} postulates that non-specific binding sites on or in aquatic macroinvertebrates can be represented by the functional groups of natural organic matter (humic acid), as described by the Windermere Humic Aqueous Model (WHAM6).

- Using quantile regression on published data from over 400 sites across three continents, complex water chemistries were condensed into a single linear function that relates the combined toxicities of metals and H^+ to the species richness of Ephemeroptera, Trichoptera and Plecoptera. The toxicity function (F_{TOX}) is the sum of the products of the bound concentration and a toxicity coefficient (α_M), for each metal or the proton. A lower threshold of F_{TOX} is defined, below which toxic effects are absent, and an upper threshold above which organisms are absent.

- The available field data, from waters affected by acid deposition and abandoned mines, permitted the derivation of parameters for four cations, with values of α_M following the sequence Cu > Zn > Al > H^+. For waters affected mainly by H^+ and Al, F_{TOX} shows a steady decline with increasing pH, crossing the lower threshold near to pH 7. Competition effects mean that toxicity due to Cu and Zn is most significant between pH 6 and pH 8.

- It should be clearly recognised that our results do not deny the environmental toxicity of heavy metals other than Cu and Zn (e.g. Ni, Cd, Hg, Pb) – it is simply that their toxicities are not expressed at the study sites used in this work.

- WHAM-F_{TOX} is a plausible model describing the toxicity of mixtures of metals and protons, based on chemical speciation concepts, and as such is a significant forward step. The results are consistent with mixture dose-response relationships in the field, supporting and extending previous conclusions based on a much smaller data set.

- Calculations with the parameterised model for different streamwater discharges suggest that in some streams, changes in the concentration of the important competing cation Ca can at least sometimes compensate for changes in heavy metal concentration. However, in systems that are only acidified (not impacted by heavy metals) low pH conditions brought about by high discharge are substantially more toxic than low-discharge conditions.

- The analysis presented here is incomplete, limited by the available published data, and further research is clearly desirable. This could include further field work, laboratory work, or the analysis of existing toxicity data. With respect to field studies, it would be especially helpful to work on systems with fewer unknown factors, or with well-known factors, so there is less reliance on quantile regression.

- Several applications of the model can be envisaged. Scientifically WHAM-F_{TOX} provides a conceptual and quantitative framework within which to evaluate mixture toxicity effects, the contributions of individual components, and the role played by "protective" cationic metals such as Mg and Ca. The model provides a means to estimate and predict actual toxicity effects in the field, as well as site-specific Environmental Quality Standards. It could be useful to guide remediation activities, including cost-benefits, which might be especially valuable for abandoned mines.

CONTENTS

1. INTRODUCTION

1.1 Background and justification

In the project *Environmental Quality Standards for trace metals in the aquatic environment* (Bass et al., 2008), the need arose to evaluate the combined toxic effects of several cationic components of natural waters, namely protons, aluminium and "heavy metals", while also taking into account the possible alleviating effects of "protective" metals such as Mg and Ca. The principal measure of toxicity in the field used in this work was the species richness of streamwater benthic macroinvertebrates. One approach was to apply a model - given the preliminary name of Toxicity Binding Model (TBM) - based on the estimated extent of binding of cations by the organisms, the idea being that this would take into account the competition effects that are inevitably operating in all field systems.

We used the TBM to describe macroinvertebrate data (OE: observed / expected species richness) in 30 metal-contaminated streams in northern England. The parameterised model explained 72% of the variance in OE, with significant contributions from H^+, Al, Zn and Cu, although in the studied waters Cu was not strongly represented. The model was formulated so as to define a threshold value of F_{tox}, above which metal effects are seen.

If the TBM could be parameterised more widely, with coverage of more metals, it might be a very useful way to describe metal effects in the environment, and to estimate the contributions of different metals under different circumstances. The model could be used to evaluate the effects of metal mixtures in specific (point-source) exposures, in diffuse inputs, for example from atmospheric deposition, and to evaluate the effects of abandoned mines. In principle it could also be extended to soils and marine systems. The project described here was conducted in order to test the model more fully, using published data from other field sites around the World.

In the previous study in northern England we used species diversity metrics derived from the River Invertebrate Prediction and Classification System (RIVPACS; Clarke et al., 2003; Wright, 2000), but this could not readily be applied to sites located other countries or continents, and so we adopted a simplified variable, the species numbers of Ephemeroptera, Plecoptera and Trichoptera (EPT). This is a widely used measure of stream ecological status, and is usually available from field studies.

Although this project was motivated by interest in the possible ecological effects of cationic heavy metals such as Ni, Cu, Zn, Cd and Pb, the previous results showed the clear need also to take into account H^+ and Al, both of which can exert toxic effects in the field. Therefore as well as analysing field data obtained from streams affected by abandoned metal mines, we also used data from studies of streams affected by acid deposition, where any toxic effects were predominantly or entirely due to H^+ and Al.

1.2 Wider context and scientific approach

Assessment of the toxic effects of metals and protons in the environment in general and freshwaters in particular would benefit from an ability to deal with mixtures. Despite this, recent books on metals contamination of aquatic systems, give scant treatment to the topic of mixture toxicity (Luoma & Rainbow, 2008; Adams & Chapman, 2007). To interpret the results of laboratory single metal toxicity studies, the concept of multi substance Potentially Affected Fractions has been developed. This combines results from experiments with single toxins to describe mixtures, assuming additivity (De Zwart et al., 2006). The Biotic Ligand Model has been applied in laboratory studies of metal mixtures, by assessing competition for binding at the biotic ligand between toxic metals (Zn, Cu, Cd) and calcium (Kamo & Nagai, 2008). Expanding these approaches to create predictive models for the full range of components in environmental systems would be a formidable task. A pragmatic means of

combining single-metal Environmental Quality Standards (EQS) for the field assessment of mixtures is through the use of Cumulative Criterion Units (Clements et al., 2000), calculated as the sum of the ratios of the concentration of each metal to its individual EQS. However, this only predicts a standard value, not a dose-response relationship, and as applied to date does not take account of chemical speciation and bioavailability.

A second issue in toxicity assessment is the need to demonstrate that estimates based on laboratory data apply in the field (Luoma & Rainbow, 2008). While this has been researched quite extensively for protons and aluminium in relation to acidification (Gensemer & Playle, 1999), results for "heavy metals" are sparse. This is clearly important to establish the credibility of EQS, to develop remediation measures, and more generally to understand ecological damage.

In the work presented here, we took a field-based approach to address both issues by developing a model, WHAM-F_{TOX}, that describes the speciation and bioavailability of mixtures and uses observed ecological effects in the field for parameterisation. We used the Windermere Humic Aqueous Model (Tipping, 1994, 1998) to calculate both aqueous chemical speciation and the non-specific accumulation of protons and metals in or on aquatic macroinvertebrates. As an ecological response variable we employed macroinvertebrate species richness, specifically of Ephemeroptera, Plecoptera and Trichoptera. The EPT index is routinely employed as a measure of biological integrity and is sensitive to a wide range of stressors (Plafkin et al, 1989). It was appropriate for the present work in view of data availability, established sensitivity, widespread distribution, functional importance in stream ecosystems and sufficient variability to indicate graded responses to metals. We applied the model to published field chemistry and EPT data from over 400 individual sites, studied with respect to either acidification or the impacts of heavy metals from abandoned mines.

Although the analysis of field data is attractive because it addresses the problem directly, a drawback is that EPT species richness may be reduced by environmental factors other than cation toxicity. Such factors may be biotic (competition, predation, dietary exposure), physical (water flow, suspended sediment), or chemical (nutrients, anionic toxicants such as arsenic, organic pollutants). Furthermore, benthic macroinvertebrates may be in contact with hyporheic groundwater, with a different chemical composition to that of the main streamflow, and may experience variations in chemistry during hydrological events. However, such factors are not widely quantified, and for our analysis are therefore "unknown". To take unknown factors into account in assessing metal and proton toxicity, we performed quantile regression modelling (Cade & Noon, 2003), which has previously been applied to assessing organism responses to metals in freshwaters, but without including chemical speciation (Pacheco et al., 2005; Linton et al., 2007; Crane et al., 2007).

2. OBJECTIVES

1. Review available data that relate stream metal concentrations, speciated with WHAM, to macroinvertebrate diversity.

2. Parameterise the TBM with all available published data, and determine whether the model can provide a unique toxicity function covering all sites and metals.

3. Examine the implications for model predictions of short-term variations in streamwater chemistry.

3. THEORY

We assume that organisms in the field accumulate metals and protons by binding at non-specific ligand sites (i.e. sites not necessarily involved in toxic action) exposed to the surrounding solution. We represent these sites by the array of binding sites postulated for humic acid in WHAM. They comprise monodentate, bidentate and tridentate binding sites comprising oxygen-containing ligands, together with less-abundant sites where binding is also influenced by the rarer "soft" ligand atoms, nitrogen and sulphur. Partial justification for the use of WHAM comes from previous work showing good agreement between its predictions and measured trace metal accumulation by aquatic bryophytes (Tipping et al., 2008). Admittedly, the element stoichiometries of invertebrates differ from those of both plants and humic substances, due to their higher nitrogen contents. However, under the relatively high cation loadings associated with cation toxicity, the abundant oxygen-containing ligands are likely to dominate. The function of WHAM in the present context is primarily to deal with binding at such sites on the invertebrates. This bold approximation provides a major advantage in addressing mixture effects in the field because the chemically-consistent parameterised WHAM readily provides estimates of non-specifically bound metals and protons, taking into account the multiplicity of competition effects that must operate. We suggest that, at steady-state, the externally bound metal and proton contents determine the "metabolically available" cation contents of the organisms, as defined by Rainbow (15). The non-specific binding does not in itself elicit a toxic response, but it represents bioavailability.

The combined toxicity of the cations is quantified by a linear toxicity function;

$$F_{TOX} = \sum \alpha_M \nu_M \tag{1}$$

where ν_M is the amount of metal bound (mmol g^{-1}), α_M is the toxicity coefficient, and M refers to each metal (including H$^+$). The toxicity coefficients relate the amount of bound metal to its individual toxic effect. Note that the model does not depend upon predicting absolute values of ν_M, only their relative amounts; thus it is not necessary to equate the total binding site densities of macroinvertebrates with those of humic acid. Equation (1) condenses complex water chemistries into a single linear variable (F_{TOX}) intended to predict toxicity. Whilst we consider competition for accumulation through the speciation modelling, for simplicity we assume that the contributions of bound cations to F_{TOX} are additive. Future developments could potentially include more complex relationships.

WHAM-F_{TOX} is similar to the Biotic Ligand Model (BLM) in that it includes the organism as a reactant in a conventional chemical equilibrium system. However in WHAM-F_{TOX} the accumulation of reactants (H$^+$ and metal cations) by the organism is non-specific, whereas in the BLM, toxicity depends upon the extent of occupation of a key site (the biotic ligand). In terms of parameterisation, the BLM requires specific equilibrium constants for the toxic metal or metals, and competing cations, including protons, derived from the results of laboratory experiments. Extension of the BLM to deal with mixtures in the field would require a major research effort to gather the necessary data, a problem which we finesse by using the pre-existing WHAM speciation code to estimate non-specific binding in WHAM-F_{TOX}.

We assume that all macroinvertebrate species accumulate metals to the same extent, but respond differently to the toxicity pressure expressed by F_{TOX}. Thus, sensitive species disappear from a stream at relatively low F_{TOX} while resistant ones can tolerate high values. In this way variations in species richness among different streams can be related to water chemistry. A desirable way to describe the data is with a value of F_{TOX} below which no toxic effects are observed as stated above (a lower threshold, F_{TOX-LT}), and a second value above which all species are absent (an upper threshold, F_{TOX-UT}). Thus we have the following conditions, in terms of species richness (SR_{EPT});

$$F_{TOX} \leq F_{TOX-LT} \qquad SR_{EPT} = SR_{EPT-max} \qquad\qquad (2)$$

$$F_{TOX} > F_{TOX-LT} \qquad SR_{EPT} = SR_{EPT-max} - \left(SR_{EPT-max} \times \frac{F_{TOX} - F_{TOX-LT}}{F_{TOX-UT} - F_{TOX-LT}} \right) \qquad (3)$$

$$F_{TOX} > F_{TOX-UT} \qquad SR_{EPT} = 0 \qquad\qquad (4)$$

4. METHODS

4.1 Field data

We required comprehensive data sets that included both water chemistry and EPT species richness for the same sites. We collated data from several studies reported in peer reviewed literature in Northern England (survey code NE; Tipping et al., 2008; Bass et al., 2008); Japan Agakawa River catchment (JA; Sasaki et al., 2005); Japan Hasama River catchment (JH; Iwasaki et al., 2009); Wales and Cornwall, UK (WC; Hirst et al., 2002); Wales acid water survey (WA; Stevens et al., 1997); Scotland and Wales acidification study (SA; Kowalik et al., 2007); and three studies in Colorado, US (CU; Mize & Deacon, 2002; Deacon et al., 2001; CE; Colorado EMAP report; and CC; Clements et al, 2008), totalling 397 individual locations. We also obtained data from the USGS National Water-Quality Assessment Program (NAWQA; downloaded from http://infotrek.er.usgs.gov/nawqa_queries/index.jsp). This archive yielded data from 15 locations across 5 survey programmes (Table S1). All 412 sites were either headwaters or other low order streams, with chemistry and SR_{EPT} data summarized in Table S2. We obtained approximately half of the data from surveys designed to investigate mine-affected sites, and the other half from surveys of sites potentially affected by acid atmospheric deposition. Mine surveys were not chosen on the basis of the extracted metals and pollution at some of the sites is as a result of mining of elements not considered in this work (e.g. Ag, Au). The data include a number of "reference" sites, not impacted by cationic toxins. Some chemical variables were missing from the data sets and values were therefore estimated (see Table S2) to attempt to improve the WHAM predictions of bound metals, but the results were hardly affected. Where we refer to a site we mean a specific location on an individual stream; the term "survey" refers to a geographical region covering a set of individual sites.

4.2 Chemical speciation calculations

The WHAM6 code (Tipping, 1998) was used to calculate the chemical speciation of streamwaters, with measured total concentrations of solution components, and measured pH, as inputs. Concentrations of dissolved organic matter (DOM) were based on measured [DOC], assuming DOM to be 50% carbon and that 65% of it is active with respect to cation binding (Tipping et al., 2008). In each case, the activity of Al was calculated from the measured total filtered concentration and also from the generalised equation derived by Tipping (2005), Eq. 5;

$$\log a_{Al^{3+}} = -10.27 + 3.26 \cdot pH - 0.49 \cdot pH^2 \qquad\qquad (5)$$

and the lower of the two values was adopted. This avoided over-estimation of Al activity in filtrates containing colloidal forms of the element. The activity of Fe(III) was calculated from the empirical equation of Lofts et al. (2008), Eq. 6;

$$\log a_{Fe^{3+}} = 2.93 - 2.70 \cdot pH \qquad\qquad (6)$$

We ran the model for two scenarios, one where the charges were forced to balance by adjusting the concentrations of Ca^{2+} and SO_4^{2-}, and another where no adjustment was made. The same metal speciation results were obtained in the vast majority of cases, with Zn being the metal with the greatest sensitivity. In approximately 10% of cases the Zn varied by 25% or more (compared to 0 - 1% of cases for the other metals). The ν_{Zn} value (Table 2) would be 13% lower without the charge balancing applied. Other ν_M values would not be significantly changed (see Supporting Information).

Because DOM in the stream waters was represented only by fulvic acid, the humic acid component of WHAM was available as a proxy for binding to macroinvertebrates. We included a concentration of humic acid in all speciation calculations at a level low enough not to affect the calculated chemical speciation of the water, thereby obtaining values of ν_M (mmol g^{-1}) for metal and proton accumulation on or in the organisms.

4.3 Fitting the data / quantile regression

Quantile regression (QR) has previously been used to integrate chemical and biological data for individual toxicants (Pacheco et al., 2005), but has not been applied to the toxicity of mixtures. An advantage of the QR approach in modelling heterogeneous variations in response distributions, is that there is no requirement to specify how variance changes are linked to the mean (*10*). It differs from least squares regression in that it results in estimates approximating either the median or other quantiles of the response variable rather than the conditional mean. Quantiles can be estimated by an optimisation function that minimises the sum of weighted absolute deviations (Equations 7 and 8), where the weightings are based on asymmetric functions of τ (this denotes the quantile and has values from 0 to 1).

$$\sum_{i}^{n} f(y_i - \xi(x_i, \beta)) \tag{7}$$

$$f(y - p) = \begin{cases} \tau(y - p) & y \geq p \\ (1 - \tau)(p - y) & y < p \end{cases} \tag{8}$$

Here y is the observed SR_{EPT} and $\xi(x_i, \beta)$ represents the model as defined in equations (1 – 4), this model provides a prediction (*p*). If the 90th quantile is modelled (τ = 0.9), 90% of the values of *y* are less than or equal to the specified function of *x* (F_{TOX}). For data that do not show the ideal response, unmeasured factors (see Introduction) become limiting and this increases the heterogeneity of organism response with respect to the measured factors included in the regression model (*10*). Unmeasured factors will result in a response that is lower than the ideal response and data affected by unknown factors therefore lie below the ideal response line (in this case the 90th quantile).

The quantile regression and parameter fitting was performed using the Solver function in Excel. Values that Excel could optimise were the toxicity coefficients (α_M), the F_{TOX} thresholds (F_{TOX-LT} and F_{TOX-UT}) and the maximum value for species richness ($SR_{EPT-MAX}$). Different starting values were applied to check there were not multiple minima for the optimisation. Providing that the values were not initially set to zero, that F_{TOX-UT} was set at a higher value than F_{TOX-LT} and that the initial estimate for $SR_{EPT-MAX}$ was between 10 and 28, the model always converged to give consistent values. We confirmed the significance of the contribution to F_{TOX} of each of the four metals that we were able to characterise, by performing t-tests. Uncertainties in the model parameters $SR_{EPT-MAX}$, F_{TOX-UT}, F_{TOX-LT}, $\square_{Al}$, $\square_{Cu}$ and $\square_{Zn}$ were estimated by bootstrapping, sampling the dataset with replacement 2000 times. The median, 13.6%ile and 86.4%ile of the distribution of each parameter were calculated; the range enclosed by the 13.6% and 86.4%iles of the distribution is a nonparametric equivalent of taking ±1 standard deviation of a normal distribution.

5. APPLICATION TO FIELD SITES

Values of α_M were extracted from the model output for protons and the following metals; Al, K, Ca, Fe, Ni, Cu, Zn, Cd, Pb (ranges shown in Figure A2). In modelling the best fits for the quantile regression, only the values of α_M for H^+, Al, Cu and Zn were appreciably greater than zero, indicating that, for the sites modelled, only these four components contributed significantly to overall toxic effects. Because only relative values are required, we fixed α_H at 1.0 and re-fitted the model to produce the results of Table 2. Figure 1 shows the observed SR_{EPT} values plotted against calculated F_{TOX}, together with the 90th quantile line. The lower threshold (F_{TOX-LT}) value of 2.26 means that for F_{TOX} greater than this value, toxic effects due to some combination of H^+, Al, Cu and Zn operate. Data that plot below the model line indicate those sites where unknown factors (see Introduction) are decreasing the species richness further than the toxic cations alone. The data show no systematic differences across the continents (i.e. biogeographic regions) included in the study (Figure A3).

We chose to model the 90th quantile, as estimates of model parameters for more extreme quantiles may have a greater uncertainty than other estimates. Extreme quantiles are also more sensitive to outliers (Pacheco et al., 2005). To test the effect of the quantile value, we additionally optimised the fitting parameters based on fitting the model to the 80th and 95th quantiles and compared the results to the 90th quantile values. Increasing the quantile results in changes in the model parameters of 20-47%, whereas decreasing the quantile results in maximum deviations of <9%. This demonstrates the greater uncertainty at larger quantiles and supports our decision to model the 90th quantile.

Simple conclusions are not readily drawn from the model outputs because of the multiplicity of interactions that determine values of F_{TOX}. Each water sample has a unique chemical composition and therefore different combinations of free ion activities, governed principally by the total concentrations of the potentially-toxic metals (including protons), other competing metals (Mg, Ca), pH and DOM. Competition effects in binding to the macroinvertebrates also have to be considered. However, by plotting F_{TOX} against pH (Figure 2), some general features can be identified. Figure 2A shows the results for the field sites. Figures 2B and 2C show calculations with the parameterised model, for idealised Ca titrations of initially acid waters, with and without Cu or Zn.

Consider first the waters from the acidification surveys that are calculated to be affected mainly by H^+ and Al (open circles in Figure 2A, long dashes in Figures 2B and 2C). At low pH, F_{TOX} is considerably higher than the lower threshold. It falls with increasing pH, only passing F_{TOX-LT} at about pH 7. Thus the model predicts that even in waters uncontaminated with heavy metals, species diversity is less than maximal for waters in the acid-to-neutral range. If Al were completely absent, then H^+ alone would exert toxic effects at pH < 6 (dotted line in Figure 2B). The contributions of H^+ and Al to F_{TOX} vary with pH (Figure 2C), Al being dominant in the pH range 5 to 6.5. Comparison of the plot for H^+ alone (Figure 2B) with the H^+ contribution plot of Figure 2C shows the competition effect of Al.

Significant contributions to F_{TOX} from Cu and Zn occur in some of the cases shown by filled triangles in Figure 2A, which represent the mining surveys. The magnitude of the deviations from the H^+-Al trend caused by Cu and Zn depends not only upon their concentrations and speciation in the streamwater, but also on the concentrations of competing cations. Thus they exert little influence at low pH because even when they are present at high concentrations, proton competition prevents ν_{Cu} or ν_{Zn} attaining values sufficient to produce significant toxic effects. This can be seen on Figure 2A, where the five mine survey sites at the top left of the plot simply extend the H^+-Al trend. On the other hand, at high pH, competition by Ca and Mg comes into play, and also complexation by carbonate species and hydroxyl ion. These effects reduce the binding of Cu and Zn by the macroinvertebrates, decreasing ν_{Cu} or ν_{Zn} and thereby decreasing F_{TOX}. Hence, the toxic effects of Cu and Zn are greatest between pH 6 and 8 (Figures 2A and 2B).

Table 1. Minimum, mean and maximum values of ν_M obtained from the WHAM modelling for each data set (units of mmol g^{-1}).

Survey	Sites	H Min	H Mean	H Max	Al Min	Al Mean	Al Max	Cu Min	Cu Mean	Cu Max	Zn Min	Zn Mean	Zn Max
US	15	0.95	1.10	1.18	0.07	0.14	0.32	0.00	0.02	0.13	0.00	0.05	0.42
CE	78	0.52	1.25	2.43	0.01	0.27	0.83	0.00	0.05	1.23	0.00	0.06	0.58
CU	13	0.84	1.21	2.89	0.07	0.23	0.62	0.00	0.04	0.16	0.00	0.31	1.08
CC	21	1.11	1.18	1.44	0.12	0.27	0.88	0.01	0.03	0.08	0.00	0.00	0.00
JH	6	0.95	1.10	1.19	0.09	0.16	0.33	0.00	0.02	0.04	0.02	0.16	0.33
JA	4	2.89	3.09	3.35	0.45	0.54	0.62	0.00	0.00	0.00	0.00	0.00	0.00
NE	33	0.80	1.23	2.51	0.03	0.37	0.88	0.00	0.01	0.12	0.00	0.17	0.92
WC	51	0.66	1.32	2.37	0.01	0.45	0.85	0.00	0.07	0.98	0.01	0.13	1.04
SA	89	1.06	1.74	2.96	0.09	0.52	0.95	0.00	0.00	0.02	0.00	0.02	0.10
WA	102	1.15	1.40	2.60	0.14	0.68	0.96	0.00	0.01	0.03	0.00	0.01	0.13
All	412	0.52	1.46	3.35	0.01	0.36	0.96	0.00	0.03	1.23	0.00	0.09	1.08

Table 2. Parameters from the best fit using the 90th quantile, with results of the significance testing for the $\square_M$ values and errors (standard deviations) estimated by bootstrapping

Parameter	Values	p
$SR_{EPT-MAX}$	23.0 ($\pm$ 2.0)	-
F_{TOX-UT}	5.10 ($\pm$ 0.65)	-
F_{TOX-LT}	2.26 ($\pm$ 0.48)	-
α_H	1[a]	< 0.001
α_{Al}	2.06 ($\pm$ 0.51)	< 0.001
α_{Cu}	3.45 ($\pm$ 0.63)	0.089[b]
α_{Zn}	2.74 ($\pm$ 0.47)	0.002

[a]The α_H value is fixed.
[b]When the acid waters surveys (WA and SA) are excluded from the testing p = 0.049.

It is important to recognise that finding significant values of α_{Cu} and α_{Zn} does not imply that Cu and Zn are more toxic than the other heavy metals considered (Ni, Cd, Pb). The significance arises because of the high concentrations of Cu and Zn in some of the streamwaters affected by abandoned mines that have been the subject of field studies. Zinc, which is comparatively mobile, is present in a high proportion of the waters, even though it is rarely the metal being mined. High concentrations of Cu are uncommon in the present data, but in a few cases very high levels occur, sufficient to establish a value of α_{Cu} (Table 2). High concentrations of Ni, Cd or Pb did not occur in any of the waters considered here, and the highest values of ν_{Ni}, ν_{Cd} and ν_{Pb} were 0.002, 0.001 and 0.24 mmol g^{-1} respectively, appreciably lower than those of ν_{Cu} and ν_{Zn} (Table 1). In some of the surveys collated for this study, the concentrations of Cd correlate with those of Zn, although generally they are much lower; the average Cd / Zn ratio in the present data set was c. 0.002 for Zn concentrations exceeding 10 nM. In laboratory toxicity tests Cd is usually found to be more toxic than Zn (Mance, 1987). However, the ratio of Cd and Zn endpoint concentrations in comparable single-metal toxicity tests is typically 0.1, much higher than the ratio from the studies in the work presented here, and so noticeable effects of Cd are not expected from our field data.

Toxic effects of Cu and Zn observed in this collection of streamwaters arise from high concentrations of these metals occurring in an appreciable number of waters, not from high intrinsic toxicity. In other words, it is high values of v_{Cu} and v_{Zn}, not α_{Cu} and α_{Zn}, that cause the high values of F_{TOX} (see Equation 1).
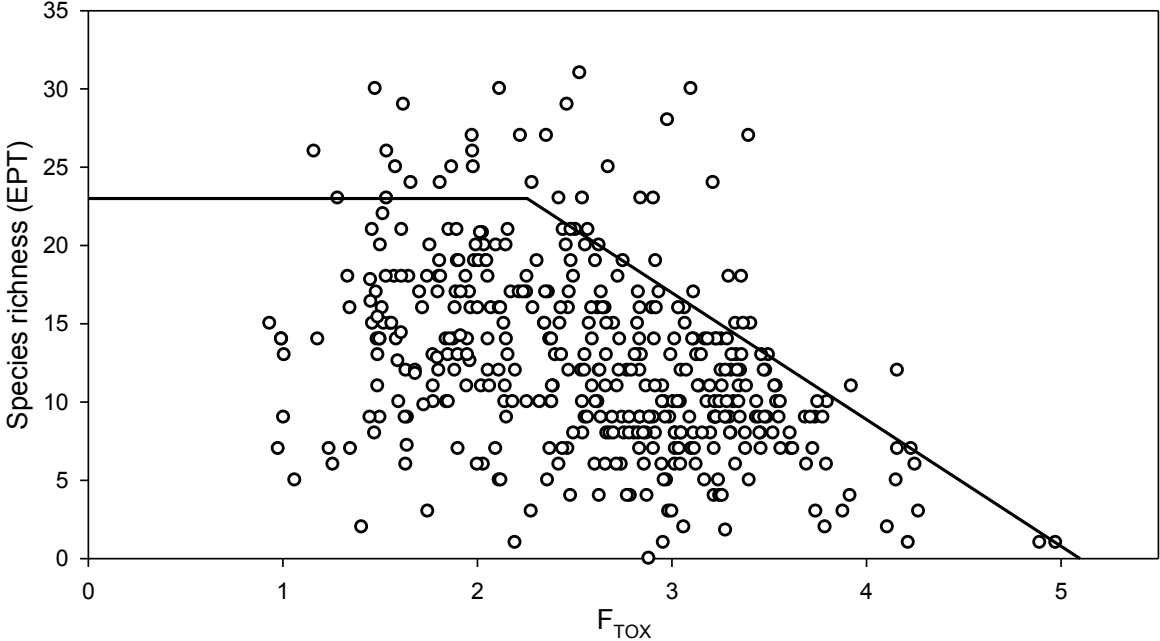

Figure 1. Results of quantile regression fitting showing the relationship between species richness and F_{TOX}. The solid line represents the best fit for the 90th quantile. Figure A3 has been included in the Supporting Information to show the results for individual surveys.
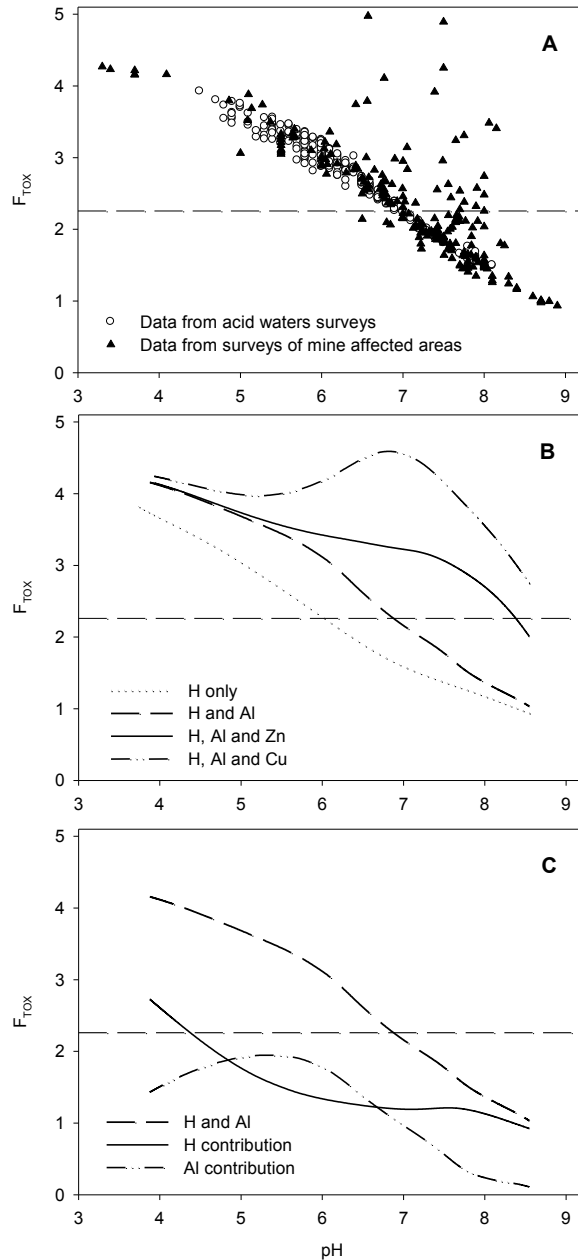
Figure 2. Variations in F_{TOX} with pH. The horizontal dashed lines represent F_{TOX-LT} and the upper limit of each plot represents F_{TOX-UT}. Panel A shows the 412 data separated into those sites surveyed for the effects of acid deposition and those of mine affected areas. Panels B and C show calculated results obtained by Ca titrations of solutions with 5 mg l^{-1} DOC, 1 mM NaCl and 0.1 mM sulphate, with and without 10 µM Cu or Zn, and assuming Al activity to be controlled by the pH relationship of Equation 5. Panel C shows the separate contributions of H^+ and Al to F_{TOX}.

6. MODELLING DISCHARGE EFFECTS

Streamwater chemistry changes with discharge, and therefore it would be expected that F_{TOX} would also vary. We explored this possibility through two examples, focusing firstly on acid systems affected by protons and Al, and secondly on a field site contaminated with Zn, for which detailed discharge and chemistry data were available.

6.1 Acid streamwaters

The sampling programme that resulted in the SA data set (Scotland and Wales acidification study, Kowalik et al., 2007) provided chemical data at both high and low flows. The results were averaged for the main analysis (Section 5), but the parameterised model can also be used to compute F_{TOX} under the two flow conditions. It is found that F_{TOX} is greater at high flow, when pH is lower and Al concentrations higher (Figure 3). In the most extreme cases, the pH at high-flow is c. 2.7 units lower than at low-flow, and the value F_{TOX} is c. 1.7 greater. These results suggest that high-discharge events are critical in acid waters.
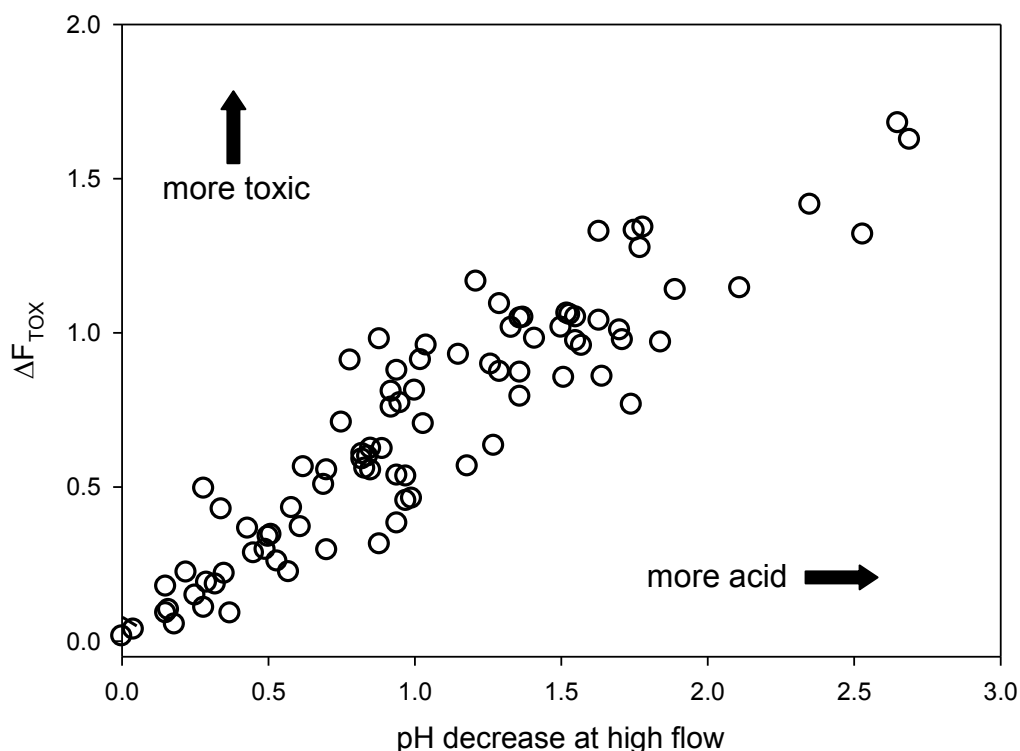
Figure 3. Changes in F_{TOX} with pH. Each point compares low- and high-flow conditions in SA dataset.

6.2 Zinc-contaminated stream in Colorado

The stream in question (French Gulch near Breckenridge) exhibits an average F_{TOX} of 3.3, more than half of which is due to Zn, the rest being contributed mainly by H^+ and Al. As shown in Figure 4 (top panel) the Zn concentration varies appreciably with discharge, falling due to dilution at high flow, and returning to high levels when discharge declines. The pH of the streamwater falls slightly at high discharge (Figure 4, middle panel), but there is sufficient buffering by Ca to prevent acid episodes. Nonetheless, Ca concentrations fall more-or-less in parallel with those of Zn.

Despite the large variations in Zn concentration, the calculated F_{TOX} hardly varies through the flow events (Figure 4, bottom panel). This is explained by the co-variance of Ca concentration, which means that competition by Ca is reduced at high flow, and so Zn binding is maintained despite the lower Zn concentration. Thus we calculate that macroinvertebrates experience almost constant toxic conditions in this stream.
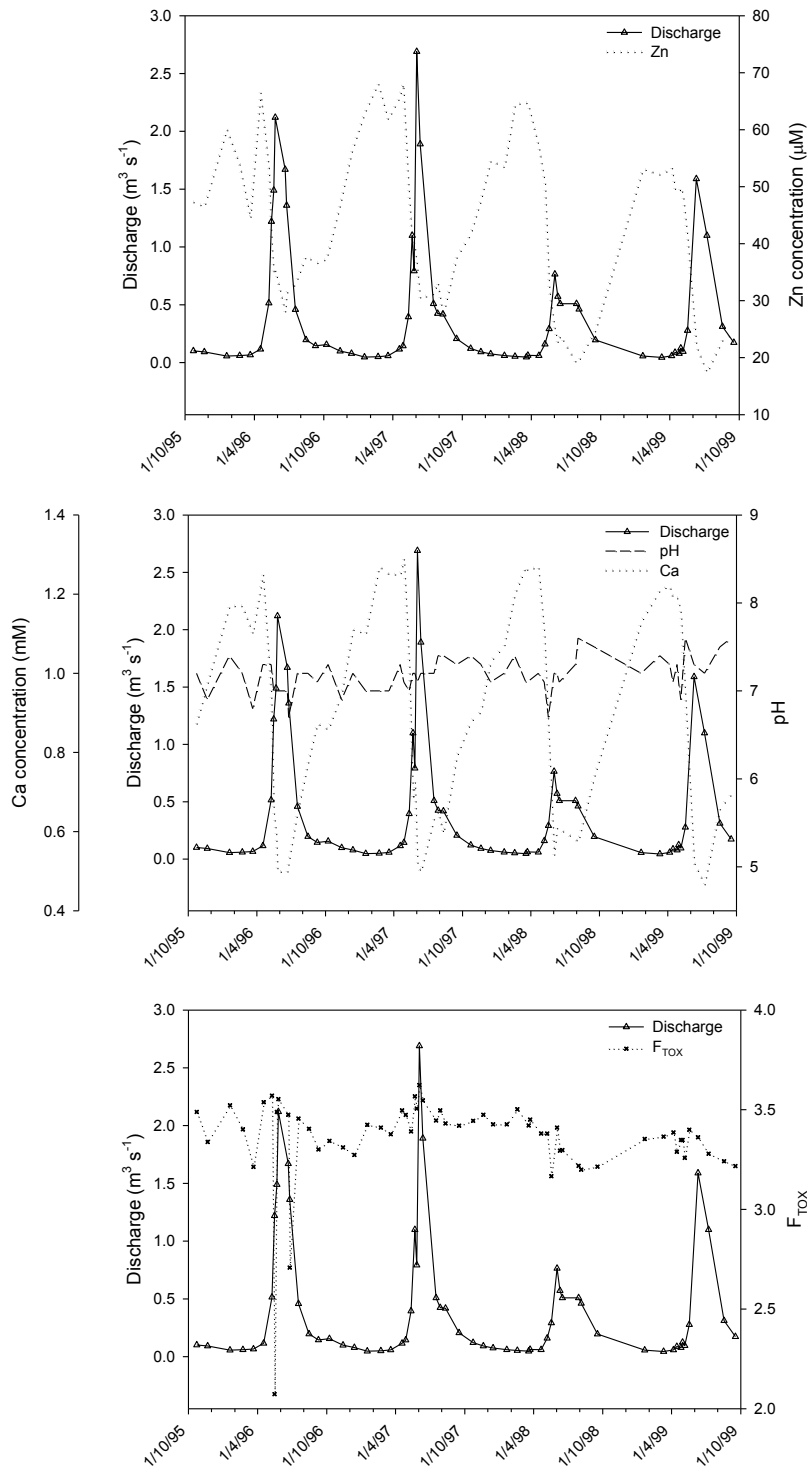
Figure 4. Effects of discharge and the associated change in water chemistry on the F_{TOX} modelling. Data are from 60 samplings of French Gulch near Breckenridge over four years, carried out by the US Geological Survey (Spahr et al., 2000).

The two low F_{TOX} values calculated for 5/96 and 8/96 are due to anomalously high reported Ni concentrations, which make the calculated Zn binding very low. (Ni is not modelled to be toxic because it did not show as significant in the main analysis of Section 5). Probably the Ni concentrations were incorrectly reported, and so the low F_{TOX} values are artefacts.

7. DISCUSSION & CONCLUSIONS

7.1 Evaluation of the model

WHAM-F_{TOX} is a plausible model describing the toxicity of mixtures of metals and protons, based on chemical speciation concepts, and as such is a significant forward step. By the use of quantile regression, we have parameterised the model using EPT species richness as a variable that responds to proton and metal toxicity, as well as to other (unknown) environmental factors. The results are consistent with mixture dose-response relationships in the field, and they support and extend the conclusions of Bass et al. (2008) that field effects can only be interpreted properly if mixtures and competition are taken into account.

A corollary of the modelling approach is the difficulty of isolating the toxic effect of any single metal in terms of dissolved concentrations. Only by considering the bound amounts, combined with the toxicity coefficients, can the contributions of individual toxicants be determined. This makes chemical sense, but assumes that toxic effects within the organism are additive.

The calculations of discharge-dependent F_{TOX} values (Section 6) suggest that in well-buffered streamwaters changes in the concentration of Zn – specifically dilution at high flow – may be compensated for by changes (decreases) in the concentration of a key competitor, in this case Ca. As a result, the value of F_{TOX} hardly varies. The same general trend would be expected for Cu, but because it binds to natural organic matter more strongly than does Zn, the compensatory competitive effect of Ca would be less, and so the higher Cu concentration a low flow would mean a higher F_{TOX}, i.e. more toxic conditions.

However, when increases in discharge bring about decreases in pH, F_{TOX} increases markedly (Figure 3), indicating that in acid streams the organisms are under much greater toxic threat during high-discharge events.

7.2 Possible developments

The analysis presented here is incomplete, limited by the available published data, and further research is clearly desirable. This could include field work on streams or lakes with a wider range of chemistries, the use of additional ecological variables, for example diatom and zooplankton diversity, or individual organism responses, and consideration of time- and flow-dependent water chemistry. It would be especially helpful to work on systems with fewer unknown factors, or with well-known factors, so there is less reliance on quantile regression.

It would be interesting to compare the calculated values of ν_M with measured body burdens for different macroinvertebrates. Some preliminary work on this was performed in the study of Bass et al. (2008) with encouraging results, but a wider study could be useful.

The extraction of values of α_M from field data is dependent upon finding sufficient stream sites where the metal in question is present at toxic levels. For streams affected by acidification and abandoned mines, as studied here, the accessible cations are restricted to just four, H^+, Al, Cu and Zn, even though data from more than 400 sites have been used in the analysis. To parameterise the model for other metals, a more targeted field approach might be needed. It should of course be emphasised that our results do not deny the environmental toxicity of other metals (e.g. Ni, Cd, Hg, Pb) – it is simply that their toxicities are not expressed at the study sites.

An alternative to the derivation of α_M from field data is to utilise published laboratory toxicity data; it may be possible to apply WHAM-F_{TOX} to derive species-specific values for a much wider range of metal cations. Given the wealth of such data available in the literature, this

could be an efficient next step. It would also be of interest to investigate possible relationships between α_M and physiological mechanisms of toxicity.

7.3 Applications

Several applications of the model can be envisaged. Scientifically WHAM-F_{TOX} provides a conceptual and quantitative framework within which to evaluate mixture toxicity effects, the contributions of individual components, and the role played by "protective" cationic metals such as Mg and Ca. The model provides a means to estimate and predict actual toxicity effects in the field, as well as site-specific Environmental Quality Standards. It could be useful to guide remediation activities, including cost-benefits, which might be especially valuable for abandoned mines.

In applying the model it should be remembered that it has only been validated for macroinvertebrates in low-order streams. There is no guarantee that the parameters would apply to metal toxicity towards different macroinvertebrate species - or indeed other types of organism – in different kinds of water. Indeed, this would be difficult to demonstrate from field data, and may have to rely on laboratory information.

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APPENDIX / SUPPORTING INFORMATION

Table A1

USGS sites used in the US data set

Table A2

Minima, maxima and mean values for the physical, chemical and EPT values for each set of data

Examination of differences in the ν values when charge balancing was not applied.

Figure A1

Figure A1. Percentage difference in ν values when charge balancing (CB) is not applied (calculated as: ν_{NOT-CB}/ν_{CB} -1).

Figure A2

Range of ν values for a range bound components in the model.

Figure A3

Results of the quantile regression fitting showing each of the surveys plotted with separate symbols

Table A1. USGS sites used in the US data set[a].

Site code for this Work	NAWQA Study Unit Code	Station ID	Site description
US01	ALMN	03037525	South Branch Plum Creek at Five Points PA
US02	ALMN	03049646	Deer Creek nr Dorseyville PA
US03	SACR	11447360	Arcade C nr Del Paso Heights CA
US04	GRSL	10167800	Little Cottonwood Creek at Crestwood Park at Slc (1999 data)
US05	GRSL	10168000	Little Cottonwood Creek at Jordan River nr Slc (2000 data)
US06	GRSL	10172200	Red Butte Creek At Fort Douglas nr Slc UT (1999 data)
US07	GRSL	404505111480 001	Emigration Creek about 1 Mile Upstream from Mouth
US08	GRSL	404751111423 501	Emigration Creek up Killyon Canyon
US09	GRSL	10167800	Little Cottonwood Creek at Crestwood Park at Slc (2000 data)
US10	GRSL	10168000	Little Cottonwood Creek at Jordan River nr Slc (2000 data)
US11	GRSL	10172200	Red Butte Creek At Fort Douglas near Slc UT (2000 data)
US12	GRSL	403818111154 201	Beaver Creek above Kamas UT
US13	GRSL	404026111273 001	Silver Creek above Richardson Flat UT
US14	YELL	06187915	Soda Butte Cr at Park bndry At Silver Gate
US15	COOK	15294700	Johnson R above Lateral Glacier nr Tuxedni Bay AK

[a]All data are from the USGS National Water-Quality Assessment Program and were downloaded from http://infotrek.er.usgs.gov/nawqa_queries/index.jsp. Reports from all of the study units can be found at http://water.usgs.gov/nawqa/studies/study_units_listing.html.

Table A2. Minima, maxima and mean values for the physical, chemical and EPT values for each set of data (all chemistry data have units of mol/l except DOC). Footnotes include details of how some of the missing data were filled.

	T (°C)			pH			DOC (mg/l)			Na			Mg		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
US	3.7	10.1	17.8	7.3	7.8	8.4	0.2	2.7	7.0	$3.4{\times}10^{-5}$	$1.8{\times}10^{-3}$	$6.3{\times}10^{-3}$	$2.5{\times}10^{-5}$	$6.4{\times}10^{-4}$	$1.7{\times}10^{-3}$
CE	4.0	11.8	22.5	5.0	7.3	8.9	2.0	2.7	10.0	$1.0{\times}10^{-4}$	$1.0{\times}10^{-4}$	$1.0{\times}10^{-4}$	$1.0{\times}10^{-5}$	$7.5{\times}10^{-5}$	$2.3{\times}10^{-4}$
CU	3.9	10.4	14.6	3.7	6.6	8.2	0.3	4.1	9.7	$3.0{\times}10^{-5}$	$2.7{\times}10^{-4}$	$1.8{\times}10^{-3}$	$1.6{\times}10^{-5}$	$1.1{\times}10^{-4}$	$7.0{\times}10^{-4}$
CC	3.8	7.1	10.5	5.6	7.3	7.9	0.8	2.0	3.0	$6.5{\times}10^{-5}$	$1.8{\times}10^{-4}$	$9.9{\times}10^{-4}$	$2.3{\times}10^{-4}$	$3.9{\times}10^{-4}$	$5.7{\times}10^{-4}$
WC	5.7	10.7	14.6	4.9	6.7	8.2	0.6	5.8	9.7	$1.6{\times}10^{-4}$	$3.6{\times}10^{-4}$	$1.8{\times}10^{-3}$	$1.4{\times}10^{-5}$	$1.1{\times}10^{-4}$	$4.4{\times}10^{-4}$
NE	6.0	6.9	7.8	4.1	7.0	8.3	0.6	4.4	8.9	$1.1{\times}10^{-4}$	$2.1{\times}10^{-4}$	$9.7{\times}10^{-4}$	$1.7{\times}10^{-5}$	$7.6{\times}10^{-5}$	$3.9{\times}10^{-4}$
SA	10.0	10.0	10.0	4.5	6.1	8.1	0.4	5.5	26.7	$1.3{\times}10^{-4}$	$2.9{\times}10^{-4}$	$8.8{\times}10^{-4}$	$2.1{\times}10^{-5}$	$7.8{\times}10^{-5}$	$5.3{\times}10^{-4}$
WA	10.0	10.0	10.0	4.9	6.3	7.8	0.6	2.2	6.4	$1.4{\times}10^{-4}$	$2.2{\times}10^{-4}$	$3.8{\times}10^{-4}$	$2.1{\times}10^{-5}$	$5.6{\times}10^{-5}$	$2.8{\times}10^{-4}$
JA	11.4	13.0	13.7	3.3	3.5	3.7	0.6	0.6	0.7	$1.0{\times}10^{-4}$	$1.0{\times}10^{-4}$	$1.0{\times}10^{-4}$	$5.0{\times}10^{-5}$	$3.4{\times}10^{-4}$	$7.0{\times}10^{-4}$
JH	8.3	14.1	20.5	7.4	7.7	7.9	0.9	1.4	2.3	$1.0{\times}10^{-4}$	$1.0{\times}10^{-4}$	$1.0{\times}10^{-4}$	$7.8{\times}10^{-5}$	$4.1{\times}10^{-4}$	$1.1{\times}10^{-3}$

	Al			K			Ca			Cr			Mn		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
US	$5.4{\times}10^{-8}$	$4.6{\times}10^{-7}$	$1.2{\times}10^{-6}$	$6.9{\times}10^{-6}$	$6.3{\times}10^{-5}$	$1.9{\times}10^{-4}$	$1.8{\times}10^{-4}$	$1.3{\times}10^{-3}$	$4.0{\times}10^{-3}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-8}$	$4.2{\times}10^{-8}$	$3.3{\times}10^{-8}$	$8.8{\times}10^{-7}$	$7.9{\times}10^{-6}$
CE	$1.1{\times}10^{-6}$	$5.2{\times}10^{-6}$	$2.1{\times}10^{-4}$	$1.0{\times}10^{-5}$	$1.0{\times}10^{-5}$	$1.0{\times}10^{-5}$	$9.3{\times}10^{-5}$	$6.8{\times}10^{-4}$	$2.1{\times}10^{-3}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.8{\times}10^{-8}$	$2.3{\times}10^{-6}$	$2.9{\times}10^{-5}$
CU	$1.6{\times}10^{-7}$	$6.5{\times}10^{-5}$	$1.8{\times}10^{-3}$	$4.9{\times}10^{-6}$	$2.1{\times}10^{-5}$	$9.3{\times}10^{-5}$	$1.6{\times}10^{-4}$	$5.5{\times}10^{-4}$	$7.0{\times}10^{-3}$	$9.6{\times}10^{-9}$	$1.8{\times}10^{-8}$	$4.2{\times}10^{-7}$	$6.9{\times}10^{-8}$	$2.2{\times}10^{-6}$	$2.0{\times}10^{-5}$
CC		equation (5)		$1.2{\times}10^{-5}$	$1.1{\times}10^{-4}$	$7.4{\times}10^{-4}$	$4.0{\times}10^{-4}$	$1.6{\times}10^{-3}$	$6.4{\times}10^{-3}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$2.5{\times}10^{-6}$	$2.5{\times}10^{-6}$	$2.5{\times}10^{-6}$
WC	$4.9{\times}10^{-7}$	$2.4{\times}10^{-6}$	$1.2{\times}10^{-5}$	$1.5{\times}10^{-6}$	$2.6{\times}10^{-5}$	$9.3{\times}10^{-5}$	$2.9{\times}10^{-5}$	$3.3{\times}10^{-4}$	$4.4{\times}10^{-3}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$6.9{\times}10^{-8}$	$1.1{\times}10^{-6}$	$1.2{\times}10^{-5}$
NE	$3.7{\times}10^{-9}$	$4.1{\times}10^{-6}$	$5.8{\times}10^{-5}$	$3.3{\times}10^{-6}$	$1.4{\times}10^{-5}$	$5.3{\times}10^{-5}$	$1.1{\times}10^{-5}$	$2.8{\times}10^{-4}$	$8.3{\times}10^{-4}$	$3.9{\times}10^{-10}$	$3.0{\times}10^{-9}$	$9.5{\times}10^{-9}$	$3.7{\times}10^{-8}$	$1.1{\times}10^{-6}$	$2.0{\times}10^{-5}$
SA	$3.7{\times}10^{-7}$	$3.1{\times}10^{-6}$	$2.7{\times}10^{-5}$	$1.3{\times}10^{-6}$	$1.2{\times}10^{-5}$	$8.1{\times}10^{-5}$	$1.0{\times}10^{-5}$	$1.2{\times}10^{-4}$	$1.2{\times}10^{-3}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$6.5{\times}10^{-7}$	$6.5{\times}10^{-7}$	$6.5{\times}10^{-7}$
WA	$1.0{\times}10^{-7}$	$2.2{\times}10^{-6}$	$1.1{\times}10^{-5}$	$1.8{\times}10^{-6}$	$7.6{\times}10^{-6}$	$3.8{\times}10^{-5}$	$1.8{\times}10^{-5}$	$9.8{\times}10^{-5}$	$7.6{\times}10^{-4}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.8{\times}10^{-8}$	$6.6{\times}10^{-7}$	$4.0{\times}10^{-6}$
JA	$8.5{\times}10^{-4}$	$1.1{\times}10^{-3}$	$1.8{\times}10^{-3}$	$1.0{\times}10^{-5}$	$1.0{\times}10^{-5}$	$1.0{\times}10^{-5}$	$5.0{\times}10^{-4}$	$3.4{\times}10^{-3}$	$7.0{\times}10^{-3}$	$1.5{\times}10^{-7}$	$3.0{\times}10^{-7}$	$4.2{\times}10^{-7}$	$1.3{\times}10^{-5}$	$1.6{\times}10^{-5}$	$2.0{\times}10^{-5}$
JH		Equation (5)		$1.0{\times}10^{-5}$	$1.0{\times}10^{-5}$	$1.0{\times}10^{-5}$	$1.0{\times}10^{-4}$	$2.5{\times}10^{-3}$	$7.2{\times}10^{-3}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$

	Co			Ni			Cu			Zn			Cd		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
US	$9.6{\times}10^{-10}$	$2.8{\times}10^{-9}$	$2.2{\times}10^{-8}$	$1.0{\times}10^{-9}$	$1.8{\times}10^{-8}$	$5.7{\times}10^{-8}$	$1.0{\times}10^{-9}$	$2.6{\times}10^{-8}$	$8.7{\times}10^{-8}$	$1.0{\times}10^{-9}$	$1.3{\times}10^{-6}$	$1.8{\times}10^{-5}$	$1.6{\times}10^{-10}$	$2.1{\times}10^{-9}$	$1.8{\times}10^{-8}$
CE	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$9.4{\times}10^{-9}$	$9.0{\times}10^{-7}$	$3.9{\times}10^{-5}$	$6.1{\times}10^{-8}$	$1.2{\times}10^{-6}$	$1.4{\times}10^{-5}$	$4.4{\times}10^{-9}$	$6.6{\times}10^{-9}$	$2.9{\times}10^{-8}$
CU	$8.5{\times}10^{-9}$	$3.0{\times}10^{-8}$	$5.3{\times}10^{-7}$	$8.5{\times}10^{-9}$	$5.8{\times}10^{-8}$	$1.6{\times}10^{-6}$	$7.9{\times}10^{-9}$	$5.7{\times}10^{-7}$	$1.3{\times}10^{-5}$	$4.7{\times}10^{-8}$	$4.9{\times}10^{-6}$	$5.1{\times}10^{-5}$	$4.4{\times}10^{-9}$	$9.2{\times}10^{-9}$	$6.5{\times}10^{-8}$
CC	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.1{\times}10^{-8}$	$3.2{\times}10^{-8}$	$6.1{\times}10^{-8}$	$5.2{\times}10^{-10}$	$1.4{\times}10^{-9}$	$2.4{\times}10^{-9}$	$1.8{\times}10^{-9}$	$3.8{\times}10^{-9}$	$6.3{\times}10^{-9}$
WC	$6.7{\times}10^{-10}$	$3.8{\times}10^{-8}$	$5.3{\times}10^{-7}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.1{\times}10^{-8}$	$7.2{\times}10^{-7}$	$1.3{\times}10^{-5}$	$1.6{\times}10^{-7}$	$4.6{\times}10^{-6}$	$5.1{\times}10^{-5}$	$2.7{\times}10^{-10}$	$6.6{\times}10^{-9}$	$6.0{\times}10^{-8}$
NE	$7.5{\times}10^{-10}$	$3.0{\times}10^{-8}$	$8.6{\times}10^{-7}$	$1.6{\times}10^{-9}$	$6.7{\times}10^{-8}$	$1.3{\times}10^{-6}$	$2.8{\times}10^{-9}$	$2.6{\times}10^{-8}$	$1.5{\times}10^{-7}$	$2.8{\times}10^{-8}$	$7.8{\times}10^{-6}$	$1.7{\times}10^{-4}$	$1.6{\times}10^{-10}$	$1.0{\times}10^{-8}$	$1.7{\times}10^{-7}$
SA	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$2.9{\times}10^{-8}$	$2.9{\times}10^{-8}$	$2.9{\times}10^{-8}$	$1.5{\times}10^{-8}$	$1.5{\times}10^{-8}$	$1.5{\times}10^{-8}$	$2.4{\times}10^{-7}$	$2.4{\times}10^{-7}$	$2.4{\times}10^{-7}$	$8.1{\times}10^{-10}$	$8.1{\times}10^{-10}$	$8.1{\times}10^{-10}$
WA	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$2.9{\times}10^{-8}$	$2.9{\times}10^{-8}$	$2.9{\times}10^{-8}$	$1.5{\times}10^{-8}$	$1.5{\times}10^{-8}$	$1.5{\times}10^{-8}$	$2.0{\times}10^{-8}$	$1.6{\times}10^{-7}$	$2.4{\times}10^{-6}$	$8.1{\times}10^{-10}$	$8.1{\times}10^{-10}$	$8.1{\times}10^{-10}$
JA	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$1.0{\times}10^{-9}$	$4.6{\times}10^{-7}$	$9.6{\times}10^{-7}$	$1.6{\times}10^{-6}$	$7.1{\times}10^{-8}$	$1.3{\times}10^{-7}$	$1.5{\times}10^{-7}$	$6.0{\times}10^{-7}$	$7.2{\times}10^{-7}$	$9.5{\times}10^{-7}$	$4.8{\times}10^{-9}$	$1.7{\times}10^{-8}$	$3.2{\times}10^{-8}$

JH	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	9.4×10^{-10}	3.0×10^{-8}	6.9×10^{-8}	5.0×10^{-8}	2.2×10^{-6}	6.7×10^{-6}	1.2×10^{-11}	1.0×10^{-8}	3.5×10^{-8}

	Ba			Pb			Cl			NO3			SO4		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
US	1.0×10^{-9}	3.5×10^{-7}	6.9×10^{-7}	3.5×10^{-10}	2.1×10^{-9}	1.1×10^{-8}	1.4×10^{-5}	1.9×10^{-3}	7.2×10^{-3}	5.5×10^{-6}	3.3×10^{-5}	7.8×10^{-5}	7.2×10^{-5}	6.5×10^{-4}	3.4×10^{-3}
CE	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.9×10^{-8}	1.9×10^{-8}	1.9×10^{-8}	1.4×10^{-5}	1.6×10^{-4}	4.4×10^{-3}	8.1×10^{-7}	1.2×10^{-6}	4.8×10^{-6}	1.0×10^{-5}	3.2×10^{-4}	2.4×10^{-3}
CU	4.4×10^{-8}	7.6×10^{-8}	1.2×10^{-6}	2.4×10^{-9}	5.5×10^{-8}	1.5×10^{-6}	1.4×10^{-6}	3.0×10^{-4}	2.1×10^{-3}	1.5×10^{-6}	1.7×10^{-5}	3.9×10^{-5}	4.8×10^{-5}	1.2×10^{-4}	2.0×10^{-3}
CC	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	8.3×10^{-6}	2.6×10^{-5}	6.2×10^{-5}	5.6×10^{-6}	1.1×10^{-5}	1.5×10^{-5}	1.4×10^{-4}	1.4×10^{-3}	4.6×10^{-3}
WC	1.3×10^{-8}	5.1×10^{-8}	1.2×10^{-7}	1.1×10^{-10}	6.3×10^{-8}	1.5×10^{-6}	1.8×10^{-4}	4.2×10^{-4}	2.1×10^{-3}	2.0×10^{-5}	2.0×10^{-5}	2.0×10^{-5}	5.0×10^{-5}	5.0×10^{-5}	5.0×10^{-5}
NE	1.4×10^{-8}	4.1×10^{-7}	3.1×10^{-6}	5.1×10^{-10}	7.4×10^{-8}	7.5×10^{-7}	1.0×10^{-4}	2.6×10^{-4}	1.2×10^{-3}	4.1×10^{-6}	1.5×10^{-5}	5.5×10^{-5}	2.9×10^{-5}	1.1×10^{-4}	8.1×10^{-4}
SA	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	7.7×10^{-9}	7.7×10^{-9}	7.7×10^{-9}	1.5×10^{-4}	3.4×10^{-4}	1.0×10^{-3}	2.1×10^{-7}	2.3×10^{-5}	4.0×10^{-4}	1.2×10^{-5}	7.3×10^{-5}	2.3×10^{-4}
WA	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	7.7×10^{-9}	7.7×10^{-9}	7.7×10^{-9}	1.5×10^{-4}	2.4×10^{-4}	4.3×10^{-4}	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	2.5×10^{-5}	6.2×10^{-5}	2.1×10^{-4}
JA	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	2.9×10^{-8}	4.1×10^{-8}	6.8×10^{-8}	1.0×10^{-4}	1.0×10^{-4}	1.0×10^{-4}	1.7×10^{-5}	2.8×10^{-5}	3.9×10^{-5}	3.0×10^{-4}	3.0×10^{-4}	3.0×10^{-4}
JH	1.0×10^{-9}	1.0×10^{-9}	1.0×10^{-9}	6.5×10^{-12}	2.6×10^{-9}	7.9×10^{-9}	1.0×10^{-4}	1.0×10^{-4}	1.0×10^{-4}	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-4}	4.0×10^{-4}	1.0×10^{-3}

	F			EPT species richness			Number of sites for each data set
	Min	Mean	Max	Min	Mean	Max	
US	5.1×10^{-6}	1.2×10^{-5}	2.1×10^{-5}	2	13	30	15
CE	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	0	13.6	30	78
CU	2.6×10^{-6}	9.1×10^{-6}	1.1×10^{-5}	1	14.4	29	13
CC	0	2.9×10^{-5}	1.1×10^{-4}	2	14.3	21	21
WC	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	1	10.1	21	51
NE	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	5	13.7	27	33
SA	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	4	11.7	30	89
WA	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	4	13.2	31	102
JA	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	3	6.8	12	4
JH	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	5	19.3	29	6

Ni, Cu, Zn, Cd, Pb data for SA and WA (not Zn) were estimated from averages from a UK wide 'Critical Loads' project (*29-31*). Mn for SA were based on the WA average. Mn data for CC were based on the average from the CU and CE data. All other missing trace metal data were set at 10^{-9} mol l^{-1}. WC nitrate data were based on the average of the SA data. EPT data for JA are upper estimates as they are based on the average of three surveys, all other EPT data are from single invertebrate surveys only. WC, JA and JH reported total organic carbon (TOC), we assumed that these values are approximately equal to DOC and applied them without modification. The partial pressure of CO_2 was set at 0.00075 atm for all of the speciation modelling.

Examination of differences in the v values when charge balancing was not applied.

It is logical to assume that there will always be a balance of cations and anions in solution. Therefore, we applied a charge balancing routine to our WHAM modelling by adjusting Ca and sulphate. However, we recognise that this procedure requires some assumptions about how this is achieved and that it is sensible to additionally view our results without the application of charge balancing. We applied the quantile regression model to v_M values obtained from WHAM modelling where charges were not forced to balance. The v_{Zn} value decreased to 2.38 from 2.74. Changes to all other independent variables were <4%. Figure A1 shows the differences in the v_M values for the four metals considered toxic in our model. Zinc binding is greatly increased at a small number of sites and this is likely to account for the decrease in the value of the zinc toxicity coefficient.
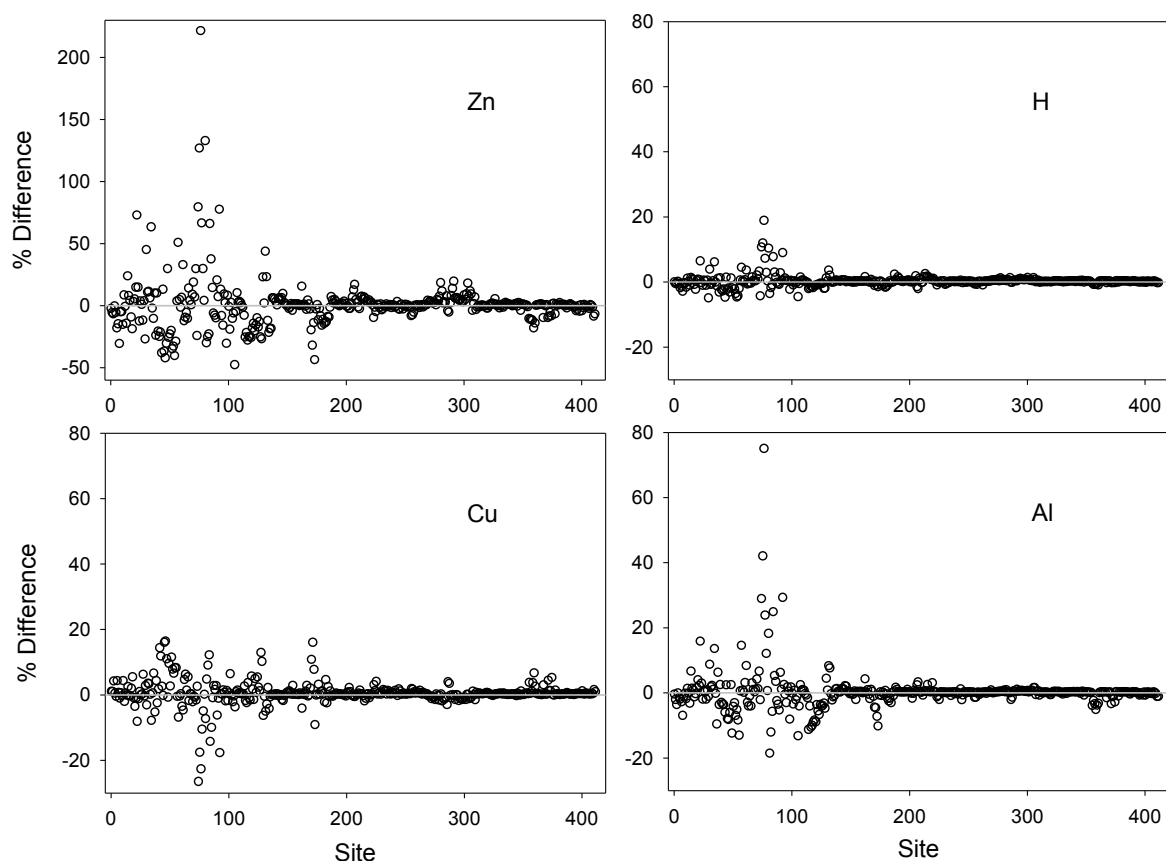

Figure A1. Percentage difference in v values when charge balancing (CB) is not applied (calculated as: v_{NOT-CB}/v_{CB} -1). Note the different y-axis scale for the Zn plot. The site number refers to the 412 sites and are ordered according to the surveys listing in Table 1 of the main manuscript. Values above the zero line indicate more metal is bound where CB is not applied, values below the zero line indicate less metal binding.
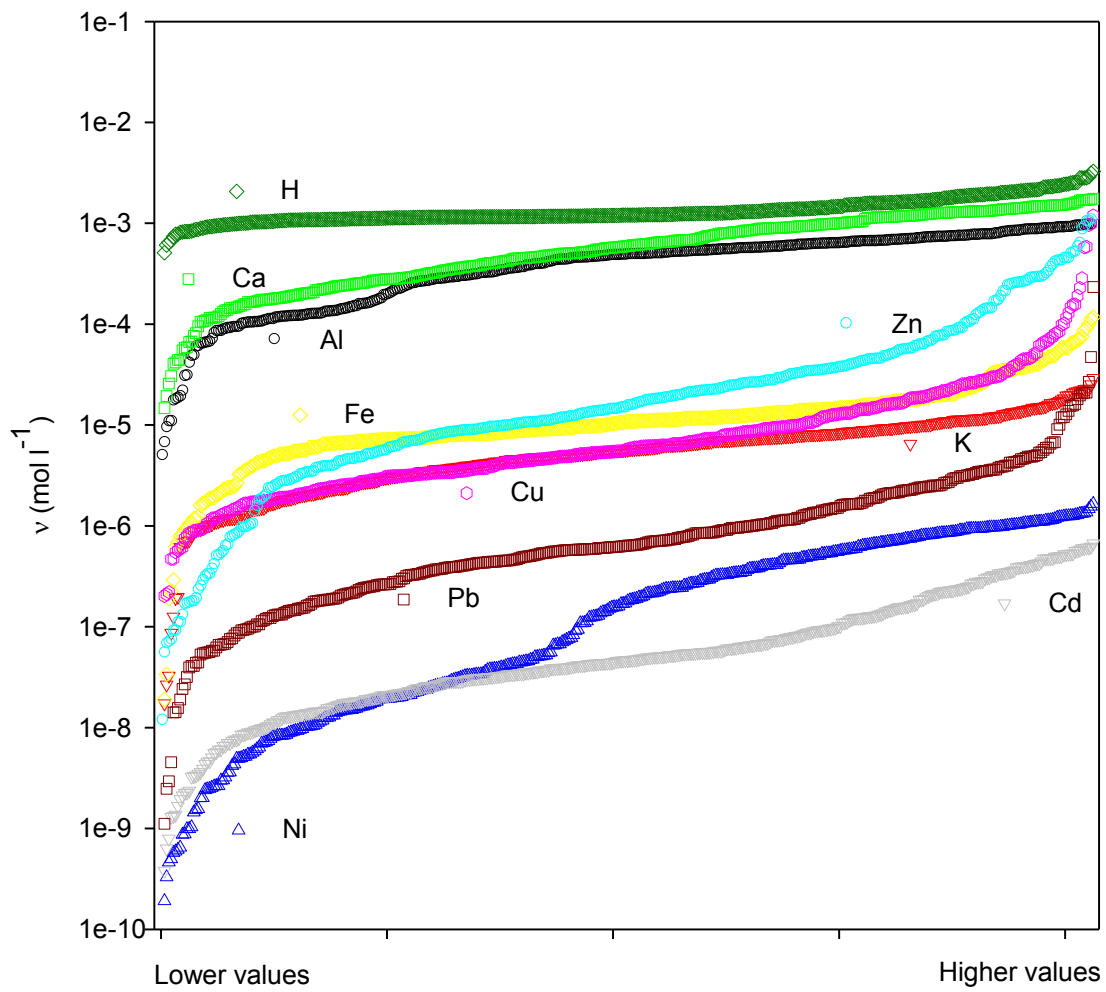
Figure A2. Range of ν values for a range bound components in the model. Note the ν values are plotted on a log scale.
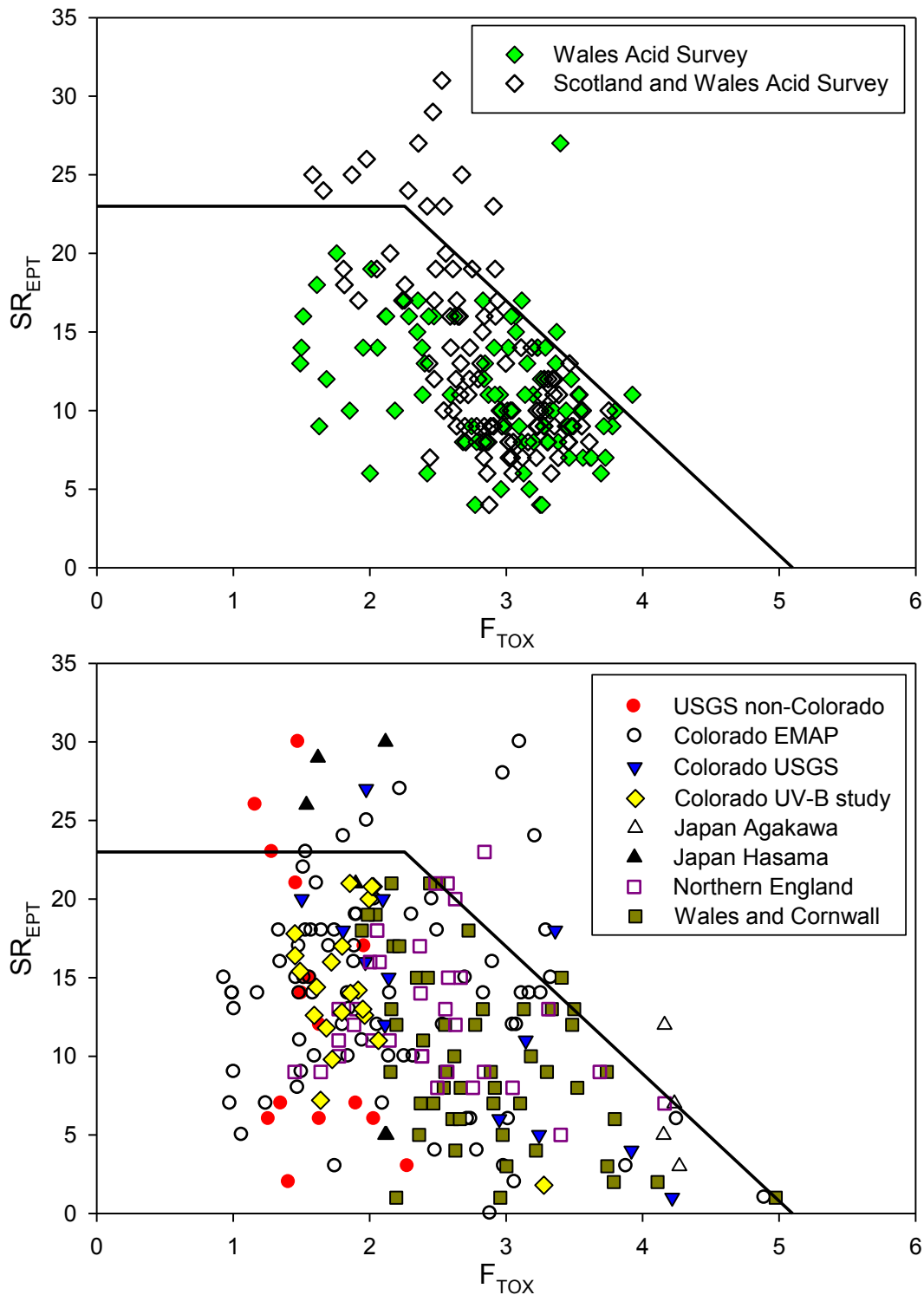
Figure A3. Results of the quantile regression fitting showing each of the surveys plotted with separate symbols. The top frame shows the two UK acidification surveys and the bottom frame all other surveys. The solid line represents the best fit for the 90th quantile based on our modelling approach.

Additional References

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