Lofts, Stephen; Criel, Peggy; Janssen, Colin R.; Lock, Koen; McGrath, Steve P.; Oorts, Koen; Rooney, Corinne P.; Smolders, Erik; **Spurgeon, David J.**; **Svendsen, Claus**; van Eeckhout, Hilda; Zhao, Fang-Zie. 2013 Modelling the effects of copper on soil organisms and processes using the free ion approach: towards a multi-species toxicity model. *Environmental Pollution*, 178. 244-253. 10.1016/j.envpol.2013.03.015

Modelling the effects of copper on soil organisms and processes using the free ion approach: towards a multi-species toxicity model

Stephen LOFTS[a,*], Peggy CRIEL[b], Colin R. JANSSEN[b], Koen LOCK[b], Steve P. McGRATH[c], Koen OORTS[d], Corinne P. ROONEY[c], Erik SMOLDERS[d], David J. SPURGEON[e], Claus SVENDSEN[e], Hilde VAN EECKHOUT[b], Fang-Zie ZHAO[c]

[a] NERC Centre for Ecology and Hydrology, Lancaster Environment Centre, Bailrigg, Lancaster LA1 4AP, United Kingdom

[b] Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, Jozef 24 Plateaustraat 22, 9000 Ghent, Belgium

[c] Rothamsted Research, Harpenden, Herts AL5 2JQ, United Kingdom

[d] Catholic University of Leuven, Division Soil and Water Management, Kasteelpark Arenberg 26 20, 3001 Leuven, Belgium

[e] NERC Centre for Ecology and Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford, Oxon OX10 8BB, United Kingdom

* Corresponding author. Tel.: +44-1524-595878; Fax.: +44-1524-61536

Abstract

The free ion approach has been previously used to calculate critical limit concentrations for soil metals based on point estimates of toxicity. Here, the approach was applied to dose–response data for copper effects on seven biological endpoints in each of 19 European soils. The approach was applied using the concept of an effective dose, comprising a function of the concentrations of free copper and 'protective' major cations, including H^+. A significant influence of H^+ on the toxicity of Cu^{2+} was found, while the effects of other cations were inconsistent. The model could be generalised by forcing the effect of H^+ and the slope of the dose–response relationship to be equal for all endpoints. This suggests the possibility of a general bioavailability model for copper effects on organisms. Furthermore, the possibility of such a model could be explored for other cationic metals such as nickel, zinc, cadmium and lead.

Keywords

soil; toxicity; copper; bioavailability; free ion approach

'Capsule'

Copper toxicity to soil organisms can be described as a pH-dependent function of the free copper ion, using a common pH dependence.

1. Introduction

Copper is a natural constituent of all soils, and in small quantities is an essential element for all plants and animals. Elevated concentrations of copper in soils can however lead to toxic effects on plants and soil–dwelling animals and hence on ecosystems as a whole (Flemming and Trevors, 1989). For this reason, ecological risk assessment of copper is an important aspect of the management of concentrations of the metal in soils.

Along with other cationic metals such as zinc and cadmium, the influence of soil chemistry on the bioaccumulation and toxicity of copper is well attested (Lexmond, 1980; Cheng and Allen, 2001). There is thus a need to develop approaches to quantify the influence of soil chemical properties on metal toxicity, in order to improve their ecological risk assessment. To date, approaches taken have been both empirical and mechanistic. In the former, endpoints from a single toxicity test, carried out in a variety of soils, are regressed against one or more soil properties believed to impact bioavailability. Such properties include soil solution pH, soil organic matter (OM) content and cation exchange capacity (CEC), and contents of mineral oxides of elements such as Fe and Mn. This type of work has been done for a number of soil organisms including barley and tomato (Rooney et al., 2006), wheat (Warne et al., 2008) and microbial processes (Oorts et al., 2006; Broos et al., 2007) for copper. The mechanistic approach centres on the Biotic Ligand Model (Paquin et al., 2002) which postulates that toxicity results from binding of specific metal species (usually the free metal ion) to a receptor on the organism (the Biotic Ligand), in competition with other solution cations such as H^+, Na^+ and Ca^{2+}. The concentration of metal bound to the biotic ligand, rather than a measurable or calculable pool of metal in the soil or soil solution, is assumed to correlate with the toxic response. The BLM was originally developed to describe the acute toxic effects of metal accumulation at the gill of fish, but has been applied to toxicity data for a number of other aquatic organisms. Some progress has been made in applying the principles of the BLM to soil–dwelling organisms: acute BLMs have been developed for soil organisms such as the earthworm *Aporrectodea caliginosa* (Steenbergen et al., 2005) and the enchytraeid *Enchytraeus albidus* (Lock et al., 2006), and the model has been applied to describe the effects of metals on plants in solution (Lock et al., 2007). Thakali and co–workers (Thakali et al., 2006a, b) have developed BLMs to predict the effects of copper on

plants, invertebrates and microbial processes, based on testing using a set of European soils of contrasting soil chemistries (Rooney et al., 2006; Oorts et al., 2006; Criel et al., 2008).

An alternative approach to considering bioavailability effects has been taken by Lofts and co–workers (Lofts et al., 2004; De Vries et al., 2007). Termed the free ion approach, this method considers the toxic effect to depend upon the free metal ion in soil solution, and also on the amounts of other solution cations that 'protect' the organism against metal toxicity. The variables considered are thus the same as would be considered by the BLM, but the expression describing the loading of the biotic ligand with toxic metal is replaced with an empirical function, and the 'biotic ligand' is not explicitly considered. The free ion approach was used to derive functions giving critical limits (risk threshold concentrations) for copper and other metals in soils directly from existing literature (Lofts et al., 2004; De Vries et al., 2007). Because of the limited nature of the available data, a number of key assumptions were made in the derivation of the critical limit functions. Such assumptions require investigation, either to confirm that they are reasonable, or to allow further refinement of the methodology. In the case of copper, datasets now exist (Rooney et al., 2006; Oorts et al., 2006; Criel et al., 2008) that are suitable for such a purpose. These datasets comprise seven toxicity tests covering a range of species and microbial processes, each carried out in the same set of soils. The soils were chosen to cover a range of key soil properties, thus making the datasets ideal for investigating metal bioavailability effects. The subset of toxicity data from the non-calcareous soils has been previously used to develop terrestrial BLMs (Thakali et al., 2006a, b). The purpose of the work presented here is to extend the free ion approach to these data and to test, for copper, the assumptions previously made in applying the approach.

2. Theory

The free ion approach is summarised in an empirical expression describing the variation of the effect concentration of a potentially toxic cationic metal in soil solution with the soil solution pH and concentrations of ‘protective’ cations. For copper:

$$\log[Cu^{2+}]_{effect} = \alpha \cdot pH_{ss} + \sum_{1}^{n} \eta_n \cdot [pC^{z+}] + \gamma_{effect} \quad (1)$$

Here pH_{ss} is the soil solution pH, $[C^{z+}]$ is the free concentration of a 'protective' cation, α, η and γ_{effect} are constants, and $[Cu^{2+}]_{effect}$ is the 'effect' concentration of the free copper ion. The subscript 'effect' refers to a constant level of toxic effect, which can be for a single species or microbial process (e.g. a no-observed effect concentration or $L(E)C_x$) or for multi–species endpoint data (e.g. a given percentile of a sensitivity distribution of species endpoints). The subscript 'effect' associated with the term γ indicates that although this term is constant at a given effect level, it will vary according to the level of effect being described. The terms α and η are assumed to be independent of effect level.

In the initial application of the theory by Lofts and co–workers (Lofts et al., 2004), two key assumptions were made. Firstly, the free concentrations of protective cations (e.g. Na^+, Mg^{2+}, Ca^{2+}) were assumed to co–vary with pH. Thus, Equation (1) was reduced to:

$$\log[Cu^{2+}]_{effect} = \alpha \cdot pH_{ss} + \gamma_{effect} \quad (2)$$

Previously employed literature data comprised chronic endpoints (no observed effect concentrations, NOECs, and 10% effect concentrations, EC10s) for plants, soil invertebrates and microbial processes. The data were rather unsystematic with respect to combinations of soil chemistry and test species, i.e. only a few test results were available for the same species across different soil types. Because of this, the data for all species were used together in a single analysis to derive the pH–dependence of free ion toxicity (the term α in Equation 2). Thus, the second assumption was that the pH dependence of free ion toxicity for all organisms and processes in the tests could be described by a single constant.

The dataset used in the present study is sufficiently comprehensive to allow the two key assumptions previously made to be tested. Firstly, concentrations of the cations Na^+, Mg^{2+}, K^+ and Ca^{2+} in soil solution can be calculated. Secondly, the pH dependence of free ion toxicity can be evaluated separately for each endpoint measured. Thus, we can formulate two central questions to be considered in the analysis of the new dataset:

1. Are the endpoint–specific dependencies of pH upon Cu^{2+} toxicity sufficiently similar to justify the use of a single, endpoint–independent value, i.e. is α similar for all endpoints?

2. Do the cations Na^+, Mg^{2+}, K^+ and Ca^{2+} exert significant protective effects against Cu^{2+} toxicity and are these all similar for all endpoints, i.e. is η_n significantly different from zero and similar for all endpoints?

1.1. The free ion effective dose model

In applying the free ion approach to these data, it would be possible to replicate in part the previous work by calculating individual toxic endpoints (e.g. EC10s or EC50s) for each test in each soil, this time expressed as free metal ion concentration, and considering how these varied with soil chemistry parameters (e.g. Oorts et al,, 2006). However, a more powerful approach is to extend the free ion approach to consider the entire dose–response curve. If we rearrange Equation (1) as follows:

$$\gamma_{\text{effect}} = \log[Cu^{2+}]_{\text{effect}} - \alpha \cdot pH_{ss} - \sum_{1}^{n} \eta_n \cdot [pC^{z+}] \quad (3)$$

it becomes clear that, since γ_{effect} is constant for a given effect level, the right hand side of the expression is also constant. Generalising to any response level, γ can be interpreted as an 'effective dose' that incorporates not only a concentration of the toxic substance, but also terms describing the effects of bioavailability. This expression can be substituted into a log–logistic dose–response equation, e.g.,

$$R = \frac{R_0}{1 + e^{\beta(D_{\text{eff}} - D_{\text{eff},50})}} \quad (4)$$

where R is the response, R_0 is the control response, β is the slope parameter, D_{eff} is the effective dose of toxicant and $D_{\text{eff, 50}}$ is the effective dose causing a 50% effect – equivalent to the ED50. If we simply substitute the effective dose term γ_{effect} in Equation 3 for the term D_{eff} then the resulting expression can in principle be fitted to dose–response curves for the same toxicity test in different soils. Fitting parameters are the terms β and $D_{\text{eff, 50}}$ in Equation 4 and the coefficients α and η_n in Equation 3. This expression will be referred to as the FRIED (FRee Ion Effective Dose) model. Although ion binding to the organism is not explicit in FRIED, the effective dose term can be related to bound metal. Mertens et al. (2007) showed that for the binding of a metal to an adsorbate in competition with H^+ and other cations, expressed by a competitive Freundlich isotherm, (Equation 5):

$$[M]_{\text{bound}} = k \cdot [M^{2+}]^{n_M} [H^+]^{n_H} \cdot \prod_{1}^{i} [C^{z+}]^{n_{C_i}} \quad (5)$$

can be rearranged to

$$\log[M^{2+}] = \left(\frac{1}{n_M}\right) \cdot \log\left(\frac{[M]_{bound}}{k}\right) + \left(\frac{n_H}{n_M}\right) \cdot pH_{ss} - \sum_{1}^{i}\left(\frac{n_{C_i}}{n_M}\right) \cdot \log[C_i^{z+}]. \quad (6)$$

Equation (6) can be simplified to

$$\log[M^{2+}] = q + r \cdot pH_{ss} - \sum_{1}^{i} s_i \cdot \log[C_i^{z+}] \quad (7)$$

where r and s_i are constants and q is given by the expression

$$q = \left(\frac{1}{n_M}\right) \cdot \log\left(\frac{[M]_{bound}}{k}\right). \quad (8)$$

Equation (7) has the same form as Equation (1) if the concentration of bound metal is constant, i.e. $q = \gamma_{effect}$ and $r = \alpha$. Equation (8) can be rearranged to show that

$$\gamma_{effect} \propto \log[M]_{bound} + X \quad (9)$$

where X is a constant. Thus, the effective dose term can be related to a conceptual quantity of metal bound to uptake sites on the organism.

The FRIED concept has been previously applied to field data. Spurgeon et al. (2006) showed that an effective dose combining the zinc free ion and pH was a better descriptor of zinc effects on *Lumbricus rubellus* reproduction in a set of field–contaminated soils than total, solution or free ionic zinc. The protective effect of the hydrogen ion was set *a priori*. Here, we will quantify the protective effect from the toxicity test data.

2. Materials and Methods

2.1. Soils dataset

Nineteen soils from across Europe were used for the toxicity testing and selected soil properties are given in Table 1 (after Oorts et al. (2006)).

Methods for the determination of soil metal and soil solution chemistry in spiked test soils are described in Rooney et al. (2006) and Oorts et al.(2006). Soil solutions were analysed for Cu, major cations (Na, Mg, Al, K, Ca and Fe) and dissolved organic carbon (DOC).

2.2. *Toxicity testing*

The toxicity tests comprised seven endpoints: two plant growth tests, two invertebrate reproduction tests and three microbial function tests, summarised in Table 2. All the tests have been described in detail elsewhere (see Table 2 for references).

2.3. Soil porewater chemistry and speciation modelling

Porewater samples were taken from all exposure soils and analysed according to the methods described by Oorts et al. (2006). Measurements of pH, dissolved organic carbon (DOC), and dissolved Cu, Na, Mg, Al, K, Ca and Fe were used here. The total copper in the exposure soils was measured after digestion with boiling aqua regia.

To apply FRIED it was first necessary to calculate the chemical speciation of the porewater solutions of the exposure soils in order to obtain concentrations of the Cu^{2+} ion and of the ions Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, Ca^{2+} and Fe^{3+}. This was done using the WHAM/Model VI model (Tipping, 1994, 1998). Input parameters were the pH, total concentrations of the ions listed above, and the soil solution concentration of fulvic acid. The latter was estimated from the DOC concentration using the assumption that the dissolved organic matter comprised 65% fulvic acid and 35% material inert with respect to chemical binding (Tipping et al., 2003). The speciation of Al and Fe(III) was modelled in one of two ways. Where the concentration of the metal exceeded the detection limit, speciation was calculated conventionally, allowing an $Al(OH)_{3\,(s)}$ or $Fe(OH)_{3\,(s)}$ solid phase to be formed if predicted. If Al or Fe(III) were not detected in solution, the speciation was predicted assuming equilibrium with $Al(OH)_{3\,(s)}$ or $Fe(OH)_{3\,(s)}$ respectively. Standard solubility constants were 8.5 and 2.7 respectively.

2.4. Application of FRIED

The dose–response equation (4) was fitted to each set of endpoint data, comprising all the responses for each toxicity test across all the soils. Prior to data fitting, the set of responses in each soil were adjusted relative to a baseline response level of 100, thus allowing responses from different soils to be modelled together. The baseline response level in each soil was set to the mean of the control response and any responses exceeding the control.

The FRIED model (Equation 3) was initially applied to each dataset using an effective dose term comprising terms for the free copper concentration and pH (Model 2). Two models (Model 0 and Model 1) were fitted as reference models. Model 0 used the

logarithm of the total soil copper concentration as the dose, while Model 1 used the logarithm of the free ionic copper. Initially, Model 2 was fitted separately to each endpoint to obtain a set of specific α values representing the protective effect of H^+ for each endpoint. The entire dataset was then fitted forcing a single value of α in order to test the assumption that a single α is a reasonable simplification. The possibility of additional protective effects due to Na^+, Mg^{2+}, K^+ or Ca^{2+} was then tested by extending the effective dose term in Model 2 to include an additional term for each ion in turn, and re–fitting the entire parameter set for each endpoint.

The Bayesian Information Criterion (BIC) (Schwartz, 1978) was used to compare the goodness of the model fits. For multiple models applied to the same dataset the smallest BIC indicates the optimum trade off between model complexity (number of parameters) and fit. We also calculated the fraction of variance explained (FVE), which is the fraction of the variance accounted for by the poorer fitting model that is then accounted for by the better fitting model. The FVE is given by:

$$\mathrm{FVE}_{j,i} = 1 - \frac{\mathrm{SOS}_j}{\mathrm{SOS}_i} \qquad (10)$$

where *i* and *j* represent the poorer and better fitting models, respectively, and SOS is the sum of squared differences between observed and calculated responses. In order to estimate the uncertainty in the parameters, fitting was done by a bootstrap method involving repeated sampling of the dataset and fitting of each sample to generate a large population of parameter sets (β, ED50, α, η_n) for statistical evaluation. Two thousand sample datasets were generated by sampling with replacement. 95% confidence intervals on parameters were taken as the 2.5%–ile and 97.5%-ile of the resulting distributions of each parameter value. Confidence intervals on the predicted dose–response curve (predicted response plotted against effective dose) were calculated by generating a predicted dose–response curve from each of the 2,000 parameter sets and taking the 2.5%–ile and 97.5%-ile of the predicted response at each value of the effective dose modelled.

2.5. Model application test

The parameterised model for *Hordeum vulgare* root elongation was applied to an independent dataset of toxicity in 17 Chinese soils (Li et al., 2010). Li and co–workers performed 5–day root elongation tests in soils that were first leached to remove excess

salts following copper spiking. We applied FRIED to predict the EC50s, expressed as total copper in the soil. Firstly, free copper ion concentrations at the EC50 in each soil were calculated using the FRIED parameters for *Hordeum vulgare*. Then, the 'geochemically active' concentrations of copper at the EC50 were calculated using the empirical function of Groenenberg et al. (2010):

$$\frac{\log\{Cu\}_{ads}}{\log[Cu^{2+}]^{0.85}} = -5.26 + 0.90 \cdot pH_{ss} + 0.89 \cdot \log\{SOM\} \quad (11)$$

where $\{Cu\}_{ads}$ is the geochemically active copper concentration in mol/g soil and {SOM} is the soil organic matter content as a %. Finally, geochemically active copper was corrected to total soil copper by accounting for fixation processes following spiking, using the model of Ma et al. (2006).

3. Results

Table 3 shows fits to Models 0, 1 and 2 for the individual toxicity tests. Model 2 ($D_{eff} = \log[Cu^{2+}] - \alpha \cdot pH_{ss}$) consistently gave a superior fit to the data than Model 1 ($D_{eff} = \log[Cu^{2+}]$). In only three toxicity tests out of seven did Model 1 explain over half the variance in the observations, while Model 2 consistently explained over half the variance in all the tests. It is worth noting that the maize residue mineralisation (MRM) test was relatively insensitive to copper within the range of applied concentrations (Figure 2), thus the R^2 was low relative to the goodness–of–fit expressed as the root mean squared error (RMSE). Excluding this test, Model 2 explained at least 65% of the observed variance in each test, while Model 1 explained 55% of the variance at best. The proportion of the unexplained variance (FVE) due to Model 1 that was explained by Model 2 was between 34% and 69% depending upon the individual test.

The BIC values for Models 0, 1 and 2 (Table 3) showed that, with the exception of MRM, Model 2 provided the best performance. Excluding the MRM results, Model 2 explained between 11% and 54% of the unexplained variance due to Model 0. Model 0 was slightly superior to Model 2 for MRM, explaining 4% of the variance unexplained by Model 2. This result may be due to the relatively small range of effects seen in the test, which is due to the relative insensitivity of the endpoint. Assuming that random errors in the measured responses are comparable in magnitude to those for the other endpoints, we would expect model fits to this dataset to be more

sensitive to such errors. Model 1 was generally inferior to Model 0, except for two tests (*Folsomia candida* reproduction and potential nitrification) (Table 3).

3.1. Modelling with global parameters

Lofts et al. (2004) assumed that the effect of pH on copper toxicity could be described by a single parameter common to all the target organisms/processes. In order to investigate this assumption quantitatively, the entire dataset was re–analysed forcing a single α across all the endpoints (Model 2a). This composite model had a total of 15 parameters (seven β values, seven $D_{eff,\,50}$ values and one α) compared to a total of 21 parameters when test-specific α values were fitted. Fitting parameters are shown in Tables 4 and 5. Forcing a global α decreased the overall R^2 and increased RMSE and BIC in comparison with the overall values calculated from the test–specific fits (Table 6). Nonetheless the decline in goodness–of–fit was not large and the imposition of a global α appeared a reasonable simplification. Furthermore, the BIC favoured Model 2a over Model 0, indicating that even with a global α, using the free ion approach significantly improved the description of the results compared with using total metal as the dose. The fitted β values mostly fell within a reasonably narrow range between unity and two, with the exception of the MRM test. This suggested a further simplification of the model by also forcing a global β (Model 2b), further reducing the number of parameters to nine. Fitting results using this model are shown in Table 6. The increase in RMSE and decrease in R^2 compared to Model 2a were marginal, and the BIC favoured Model 2b over Model 2a. Figure 3 compares the overall fits of Models 2, 2a and 2b.

3.2. Effect of pH and major cations in the effective dose

The effective dose term in Model 2 was extended to consider the effect of an additional cation as well as H^+. This model was then applied to each endpoint in turn, in each case fitting the effect of one major cation (Na^+, Mg^{2+}, K^+, Ca^{2+}) in addition to H^+, giving 28 fits in all. The entire parameter set (α, β, $D_{eff,\,50}$, η) was fitted in each case. The resulting fits were compared to the fits obtained using Model 2 by comparing BIC values. The cation was considered to exert an effect where the BIC value for the fit using the extended model was smaller than that for the corresponding fit using Model 2. Table 6 indicates whether the cation exhibited a ‘protective’ or a

315 'toxic' effect, judged from the sign of the coefficient, η, of $p[C^{z+}]$ in Equation 3. A
316 negative coefficient indicates a 'protective' effect while a positive coefficient indicates
317 a 'toxic' effect. Generally there was little systematic pattern to the effects found. Of
318 the 28 fits, an effect due to the major cation was found in 17. However, only in six of
319 these cases was the effect 'protective'; in the remaining cases, an apparent 'toxic' effect
320 of the additional cation was seen. The most consistent pattern of effect was observed
321 for Ca^{2+}, with a 'toxic' effect observed in six of the seven tests. Enhancement of
322 toxicity due to Mg^{2+} was observed in four tests and a protective effect in one other.
323 Na^{+} showed a toxic effect in only one test and a protective effect in three others, while
324 K^{+} showed no enhancing effects and protective effects in two tests only.

325 *3.3. Application to the Chinese dataset*

326 The expression for the EC50 as the free copper ion for *Hordeum vulgare*, from the
327 parameterised model, is

328 $$\log[Cu^{2+}]_{EC50} = -0.79 \cdot pH_{ss} - 2.60 \quad (12)$$

329 Li et al. (2010) measured the pH of the soils using a deionised water extraction. To
330 convert their values to pH_{ss}, we applied the expression given by De Vries et al.
331 (2007):

332 $$pH_{ss} = 1.05 pH_{H_2O} - 0.28 \quad (13)$$

333 Ten soils had pH_{ss} values within the calibration range of FRIED (pH_{ss} = 3.1–8.0);
334 therefore, the analysis was confined to these soils. Back–calculation from copper free
335 ion to total soil copper at the EC50 gave the result shown in Figure 4. The root mean
336 squared deviation in log total Cu (mg/kg) was 0.17, and nine of the ten measured
337 EC50s were predicted to within a factor of two. A small optimisation of $D_{eff,\,50}$ from
338 -2.60 to -2.42 further reduced the root mean squared error to 0.038 (Figure 4).

339 ## 4. Discussion

340 *4.1. Performance of the FRIED model*

341 FRIED was successful in describing the variability in copper toxicity across the
342 different soils. In six of the seven tests, FRIED fits were superior to those obtained
343 taking total soil metal as the effective dose. FRIED fitting confirmed a significant
344 effect of pH on Cu^{2+} toxicity, in agreement with previous work such as that by
345 Steenbergen et al. (2005) on the acute toxicity of Cu to the earthworm *Aporrectodea*

346 *caliginosa*, Thakali et al. (2006a, b) on the non-calcareous soils of this dataset, and the
347 work of Lofts et al. (2004) on deriving critical limit functions. The pH dependence of
348 Cu^{2+} toxicity has also been previously demonstrated for the plant and microbial
349 process toxicity data used here, by calculating soil–specific EC10s and EC50s
350 expressed as Cu^{2+} and regressing these against soil pH (Oorts et al., 2006; Zhao et al.,
351 2006). FRIED extends this approach by fitting a set of complete dose–response
352 curves, instead of ECx values.
353 We have tested the key assumption made by Lofts et al. (2004) that the pH–
354 dependence of free ion toxicity is the same regardless of the soil organism or process
355 under consideration. While modelling the whole dataset forcing a single α gave an
356 inferior fit in comparison to that obtained by allowing endpoint–specific α values, the
357 goodness–of–fit was not greatly poorer and the model was favoured over those where
358 protective effects of H^+ were not considered. Thus, the assumption of a global α is
359 reasonable if specific data on the α value for a given endpoint are not available, as
360 was the case in the work of Lofts et al (2004). In that work, an α value of -1.21 was
361 derived, later refined to -1.26 (De Vries et al., 2007). This is a larger dependence of
362 apparent free ion toxicity on pH than we have calculated when forcing global α values
363 in Models 2a and 2b (-0.89 and -0.94 respectively). The dataset of Lofts et al. (2004)
364 was not systematic in terms of individual endpoint measurement across different soil
365 compositions. The datasets used here are structured and comprehensive in respect of
366 measurements across a range of soil compositions, for a range of organisms and
367 processes. Thus, it is not surprising to find that the α value differs between the two
368 studies. A logical next step would be to use the global α value in an updated
369 calculation of a critical limit function for copper.
370 The term α quantifies Cu^{2+}:H^+ competition at the site of toxic action. Thus, the
371 similarity among α values for the different endpoints implies similarity in the ion
372 binding behaviour at the site(s) of toxic action on the organisms. It has been
373 suggested that the underlying mechanism of Cu toxicity is binding of Cu to thiol
374 groups in proteins and consequent damage to their structure (Letelier et al., 2005); this
375 has been noted as a reason for the sensitivity of plant ATPases to Cu (De Vos et al.,
376 1991) and thus might also be related to the well–established effects of Cu on the
377 Na^+/K^+-ATPase in animals (e.g. Lauren and McDonald, 1986)). If Cu initially binds
378 to ATPase carrier proteins or ion pumps on the cell membrane, this may account for

the apparent similarity in the binding behaviour of the uptake sites in different taxa, particularly because it is recognised that there is a high conservation of the amino acid sequence and crystal structure of known ion pumps such as metazoan Na^+/K^+-ATPases (e.g. Pressley, 1992; Ma et al., 2005). Further investigation of this phenomenon would be facilitated with knowledge of α values for other organisms, such as freshwater species. De Schamphelaere and Janssen (2006) found that the toxic effects of copper (expressed as Cu^{2+} activity) on the growth rate of two algal species (*Pseudokirhcneriella subcapitata* and *Chlorella vulgaris*)could be described as a function of pH. Their calculated slope values are compared with the slopes derived in this study in Figure 5. It can be seen that the slope for *Chlorella vulgaris* is not statistically different from four of the slopes calculated in this study, and the slope for *Pseudokirchneriella subcapitata* is not statistically different from one slope calculated in this study. Based on this analysis, we tentatively suggest that the hypothesis of a common, organism–independent slope for the pH dependence is worthy of further investigation.

Imposing a global β value as well as a global α produced a fit superior to that where endpoint–specific βs were allowed; although the RMSE was slightly inferior, the BIC indicated that this was well compensated by the smaller number of parameters (Table 5). The resulting model contains only a single species–specific parameter, $D_{eff,\,50}$, and can be used to derive a generic expression for estimation of an effect concentration of Cu^{2+} from any other effect concentration. Rearranging Equation 4 gives a general expression for the concentration of Cu^{2+} causing a given level of effect in a soil:

$$\log[Cu^{2+}]_R = \alpha \cdot pH_{ss} + D_{eff,50} + \frac{1}{\beta} \cdot \ln\left(\frac{R_0 - R}{R}\right) \qquad (14)$$

Considering two different response levels R_1 and R_2 of a given organism or process to copper in two soils having pH_{ss} values denoted $pH_{ss,\,1}$ and $pH_{ss,\,2}$, we can derive the following expression:

$$\log[Cu^{2+}]_{R1} = \log[Cu^{2+}]_{R2} + \alpha \cdot (pH_{ss,1} - pH_{ss,2}) + \frac{1}{\beta} \cdot \left[\ln\left(\frac{R_0 - R_1}{R_1}\right) - \ln\left(\frac{R_0 - R_2}{R_2}\right)\right] \qquad (15)$$

which allows one effect concentration to be estimated from the other. Using global values of α and β, this expression is potentially useful for risk assessment since it

allows an effect concentration for a given species to be calculated in a target soil, given only an effect concentration in another soil of known pH_{ss}.

The lack of consistent effects due to other cations (Na^+, Mg^{2+}, K^+, Ca^{2+}) is an interesting finding, particularly as the model fitting suggests an additional toxic effect due to these cations in some cases. Thakali et al. (2006a, b) did not find protective effects due to ions other than H^+ when applying a BLM to the subset of non-calcareous soils, other than an Mg effect on potential nitrification. Protective effects of Mg^{2+} and Ca^{2+} against Cu toxicity have been previously observed, for example by Kinraide et al. (2004) and Luo et al. (2008) for root elongation of wheat (*Triticum aestivum*) in nutrient solutions. On the other hand, Steenbergen et al. (2005) found that Mg^{2+} and Ca^{2+} did not protect the earthworm *A. caliginosa* against the acute toxicity of Cu^{2+} but appeared to contribute to the toxicity. They suggested that co–variance between H^+, Mg^{2+} and Ca^{2+} activities was at least partly responsible for these observations. Such co–variance is also observed here; soil solution ion concentrations, particularly Mg and Ca, tend to increase with increasing copper dose (Figure 6) due to competitive displacement from binding sites on the soil solids by Cu. Clearly these side effects of dosing with a soluble metal salt may be confounding a rigorous analysis of protective effects; future studies need to consider how such side effects might be minimised, for example by leaching the soil following dosing and prior to toxicity testing (e.g. Bongers et al., 2004; Oorts et al., 2007; Smolders et al., 2009; Li et al., 2010).

This study bears comparison to the work of Thakali et al. (2006a; 2006b) on the development of a terrestrial BLM for copper. In this study, we have used a soils dataset covering both non–calcareous and calcareous soils, while Thakali et al. confined their analysis to the non–calcareous soils of the same dataset. In applying the BLM to the data, Thakali and co–workers found it necessary to fix the fractional occupancy of the biotic ligand corresponding to a 50% effect, before fitting binding constants for Cu^{2+} and H^+. FRIED avoids the need to fit separate affinity parameters for the potentially toxic metal and competing ion(s) as the α parameter expresses the relative binding affinities of Cu^{2+} and H^+. FRIED has also been useful in illustrating common patterns of Cu bioavailability across different endpoints, supporting the hypothesis of a single α made by Lofts et al. (2004). This would likely be difficult to achieve using a BLM unless concentrations of metal at site(s) of toxic action were

measured. It would be highly desirable to investigate whether similar patterns in bioavailability parameters exist for other cationic metals.

The ability of FRIED to predict EC50s in the independent dataset of Li et al. (2010) largely to within a factor or two, without any optimisation, is an encouraging finding for the validity of the model and suggests its potential for use in risk assessment for prediction of toxicity as the total metal in soil, when coupled with expressions to relate the predicted toxic free ion concentration to the total or the geochemically active soil metal concentration. Here, we calculated the total soil copper from the free ion using a two–stage calculation entailing the calculation of the geochemically active metal pool. Alternative possibilities are the direct calculation of the total metal from the free ion, given a suitable empirical relationship, or the calculation of the geochemically active metal from the free ion using a speciation model.

An important difference between this study and the BLM work of Thakali and co–workers was that the latter related the free Cu ion to the total soil metal using WHAM, while this study calculated free Cu from measurements on the soil solution. The ability of the parameterised BLM to predict toxicity on the basis of total soil metal is useful for standard–setting and risk assessment purposes. However, for the purpose of optimally parameterising a toxicity model based on the chemistry of the soil solution it is likely that calculating free ion from the soil solution is more reliable than calculating it based on measurements of the soil solid phase composition.

As we have shown by application of the model to the Chinese soils dataset of Li et al. (2010), FRIED could readily be coupled to an empirical or mechanistic partitioning model to enable the link to total soil metal concentration to be made.

Acknowledgements

We thank Ed Tipping for discussions and Helen Hooper for comments on an early draft of the manuscript. This work was funded by the International Copper Association. Rothamsted Research receives grant-aided support from the UK Biotechnology and Biological Sciences Research Council.

Table 1. Selected properties of the soils used in toxicity testing

Soil	Location	pH[a]	C_{org}[b] g/kg DW soil	C_{inorg}[c] g/kg DW soil	Clay[d] g/kg DW soil	Cu[e] mg/kg DW soil
Gudow	Germany	3.0	51	0	19	2
Nottingham	UK	3.4	52	0	78	17
Houthalen	Belgium	3.4	19	0	31	2
Rhydtalog	UK	4.2	129	0	1	14
Zegveld	Netherlands	4.7	233	0	7	70
Kövlinge I	Sweden	4.8	16	0	54	6
Souli I	Greece	4.8	4.1	0	376	31
Kövlinge II	Sweden	5.1	24	0	67	8
Montpellier	France	5.2	7.6	0	82	5
Aluminusa	Italy	5.4	8.7	0	501	21
Woburn	UK	6.4	44	0	166	22
Ter Munck (Leuven)	Belgium	6.8	9.8	0	140	22
Vault de Lugny	France	7.3	15	60	365	21
Rots	France	7.4	13	149	257	14
Souli II	Greece	7.4	26	474	434	34
Marknesse	Netherlands	7.5	13	100	247	18
Barcelona	Spain	7.5	15	72	195	88
Brécy	France	7.5	15	176	485	31
Guadelajara	Spain	7.5	3.8	365	246	7

[a] measured using 0.01M $CaCl_2$.
[b] organic carbon.
[c] inorganic carbon.
[d] Clay fraction measured after removal of organic matter from soil.
[e]Measured using boiling aqua regia extraction.

Table 2. Summary of toxicity tests carried out.

Test species/process	Endpoint	Number of data points	Reference
Hordeum vulgare	Root growth	125	Rooney et al. (2006)
Lycopersicon esculentum	Shoot growth	126	Rooney et al. (2006)
Folsomia candida	Reproduction	93	Criel et al. (2008)
Eisenia fetida	Reproduction	79	Criel et al. (2008)
Potential nitrification	Inhibition	79	Oorts et al. (2006)
Maize residue mineralisation	Inhibition	132	Oorts et al. (2006)
Glucose–induced respiration	Inhibition	98	Oorts et al. (2006)

Table 3. Test–specific parameters and fitting measures for the effective dose Models 0, 1 and 2[a]. Abbreviations for the toxicity tests are: Hv ≡ *Hordeum vulgare* root elongation; Le ≡ *Lycopersicon esculentum* shoot elongation; Fc ≡ *Folsomia candida* reproduction; Ef ≡ *Eisenia fetida* reproduction; PN ≡ potential nitrification; MRM ≡ maize residue mineralization; GIR ≡ glucose–induced respiration.

Model 0: $D_{eff} = \log[Cu]_{soil}$ (mg/kg DW soil)													
Test	β			$D_{eff,\,50}$			α			RMSE[b]	R^2	$FVE_{M2,\,M0}$[c]	$FVE_{M2,\,M1}$[c]	BIC[d]
Hv	3.36	(2.70,	4.27)	2.34	(2.25,	2.42)	–			21.7	0.67	–	–	789
Le	2.96	(2.24,	4.34)	2.52	(2.40,	2.64)	–			28.5	0.49	–	–	864
Fc	1.63	(1.16,	2.19)	2.66	(2.47,	2.91)	–			25.0	0.40	–	–	613
Ef	3.88	(2.73,	6.88)	2.44	(2.34,	2.55)	–			23.5	0.60	–	–	516
PN	2.42	(1.65,	3.91)	2.67	(2.49,	2.86)	–			30.0	0.43	–	–	555
MRM	1.31	(1.02,	1.68)	3.70	(3.48,	4.04)	–			11.6	0.54	–	–	666
GIR	1.83	(1.29,	2.54)	2.89	(2.70,	3.17)	–			23.3	0.50	–	–	636
Model 1: $D_{eff} = \log[Cu^{2+}]$ (M)														
Test	β			$D_{eff,\,50}$			α			RMSE	R^2	$FVE_{M2,\,M0}$	$FVE_{M2,\,M1}$	BIC
Hv	0.62	(0.52,	0.75)	-7.06	(-7.56,	-6.57)	–			25.8	0.54	(–)	–	832
Le	0.54	(0.43,	0.71)	-6.95	(-7.56,	-6.39)	–			28.9	0.48	(–)	–	867
Fc	0.60	(0.45,	0.81)	-5.26	(-5.74,	-4.83)	–			21.9	0.54	0.24	–	592
Ef	0.39	(0.21,	0.69)	-4.99	(-5.89,	-3.64)	–			33.0	0.21	(–)	–	570
PN	0.85	(0.61,	1.32)	-5.66	(-6.19,	-5.15)	–			26.7	0.55	0.21	–	536
MRM	0.17	(0.09,	0.27)	3.05	(-0.18,	11.01)	–			15.8	0.15	(–)	–	748
GIR	0.30	(0.19,	0.43)	-3.86	(-4.86,	-2.15)	–			28.5	0.26	(–)	–	675
Model 2: $D_{eff} = \log[Cu^{2+}] - \alpha \cdot pH_{ss}$														
Test	β			$D_{eff,\,50}$			α			RMSE	R^2	$FVE_{M2,\,M0}$	$FVE_{M2,\,M1}$	BIC
Hv	1.74	(1.46,	2.18)	-2.60	(-3.14,	-2.06)	-0.79	(-0.88,	-0.70)	15.4	0.84	0.50	0.64	703
Le	1.67	(1.21,	3.19)	-1.75	(-2.61,	-0.97)	-0.98	(-1.11,	-0.83)	23.2	0.67	0.34	0.36	812
Fc	1.05	(0.87,	1.29)	-1.72	(-2.69,	-0.62)	-0.75	(-0.93,	-0.55)	17.0	0.72	0.54	0.37	545
Ef	1.93	(1.31,	3.22)	0.30	(-0.52,	1.31)	-1.18	(-1.36,	-1.02)	22.2	0.65	0.11	0.55	507
PN	2.15	(1.27,	10.03)	-2.44	(-3.59,	-1.29)	-0.63	(-0.80,	-0.45)	21.6	0.71	0.48	0.34	503
MRM	0.72	(0.55,	0.96)	2.68	(1.78,	3.74)	-1.11	(-1.24,	-0.98)	11.8	0.53	(–)	0.44	671
GIR	1.57	(1.26,	2.14)	1.07	(0.43,	1.78)	-1.15	(-1.25,	-1.06)	15.8	0.77	0.54	0.69	560

[a] Values in brackets are the 95% confidence intervals of the parameters, calculated by bootstrapping.
[b] root mean squared error in % response.
[c] Fraction of variance unexplained by Model 0 or Model 1, that is explained by Model 2.
[d] Bayesian information criterion.

Table 4. Fitted parameters for the variants of Model 2 (FRIED) where either α (Model 2a) or α and β (Model 2b) are forced to global values[a].

	Model 2a: global α								
Test	β			$D_{eff, 50}$			α		
Hv	1.66	(1.41,	2.04)	-1.81	(-2.41,	-1.51)			
Le	1.53	(1.12,	2.63)	-2.27	(-2.71,	-1.86)			
Fc	1.07	(0.89,	1.32)	-0.96	(-1.42,	-0.49)			
Ef	1.37	(0.85,	1.98)	-1.26	(-1.80,	-0.89)	**-0.89**	**(-0.95,**	**-0.82)**
PN	1.95	(1.24,	11.21)	-0.89	(-1.37,	-0.44)			
MRM	0.71	(0.57,	0.91)	1.50	(1.14,	1.62)			
GIR	1.10	(0.78,	1.47)	-0.35	(-0.83,	0.11)			
	Model 2b: global α and β								
Test	β			$D_{eff, 50}$			α		
Hv				-1.58	(-1.96,	-1.16)			
Le				-1.92	(-2.34,	-1.53)			
Fc				-0.72	(-1.14,	-0.26)			
Ef	**1.33**	**(1.21,**	**1.48)**	-0.99	(-1.36,	-0.63)	**-0.94**	**(-1.00,**	**-0.88)**
PN				-0.58	(-1.03,	-0.08)			
MRM				1.39	(1.07,	1.71)			
GIR				-0.10	(-0.50,	0.37)			

[a] Figures in brackets are the 95% confidence intervals of the parameter, obtained by bootstrapping.

Table 5. Fitting statistics for models using the different effective dose terms. Errors are calculated by combining the results of fitting to all seven endpoints. Models 2a and 2b contain one or two parameters forced to global values (see text for details).

Model	Effective dose	RMSE[a]	R^2	FVE_{M0}	FVE_{M1}	BIC[b]
0	$\log[Cu]_{soil}$	23.5	0.55	(–)	(–)	4722
1	$\log[Cu^{2+}]$	25.8	0.46	(–)	(–)	4858
2	$\log[Cu^{2+}] - \alpha \cdot pH_{ss}$	18.3	0.73	0.40	0.50	4395
2a	$\log[Cu^{2+}] - \alpha \cdot pH_{ss}$	19.5	0.69	0.31	0.43	4458
2b	$\log[Cu^{2+}] - \alpha \cdot pH_{ss}$	19.9	0.68	0.29	0.41	4442

[a] Root mean squared error in the response.
[b] Bayesian information criterion.
[c] Fraction of variance explained; see text for explanation.

Table 6. Performance of effective dose models comprising terms for pH and one of p[Na^+], p[Mg^{2+}], p[K^+] and p[Ca^{2+}]. The letters P and T indicate an improved model fit due to inclusion of the additional term, based on a lower value of the Bayesian Information Criterion for the extended model. The letter P indicates an apparent protective effect ($\eta < 0$) and the letter T indicates an apparent toxic effect ($\eta > 0$). Where no letter is shown, the additional term did not improve the model fit.

	$D_{eff} = \log[Cu^{2+}] - \alpha \cdot pH_{ss} - \eta \cdot p[C^{z+}]$			
Test	Na^+	Mg^{2+}	K^+	Ca^{2+}
Hv		T	P	T
Le		T		T
Fc	P	P	P	T
Ef	T	T		T
PNR	P			
MRM		T		T
GIR	P			T

Figures

Figure 1. Comparison of fits of Model 1 (left hand panes) and Model 2 (right hand panes) for effects on *H. vulgare*, *L. esculentum*, *F. candida* and *E. fetida*.

Figure 2. Comparison of fits of Model 1 (left hand panes) and Model 2 (right hand panes) for effects on potential nitiration rate (PN), maize residue mineralization (MRM) and glucose–induced respiration (GIR).

Figure 3. Results of fitting the data globally with single values of α and β. Observed and predicted response plotted against the standardised effective dose, $D_{eff,\,S}$ [$D_{eff,\,S} = \beta \cdot (D_{eff} - D_{eff,\,50})$]. Closed circles: *H. vulgare*; open circles: *L. esculentum*; closed triangles: *F. candida*; open triangles: *E. fetida*; closed squares: potential nitrification rate (PN); open squares; maize residue mineralization (MRM); closed diamonds: glucose–induced respiration (GIR).

Figure 4. Prediction of EC50s for copper effect on *H. vulgare* root elongation in the dataset of Li et al. (2010), using the endpoint–specific parameter set (Model 2). Solid points represent EC50s calculated by blind prediction, open points represent EC50s calculated by optimisation of $D_{eff,\,50}$. The solid line is the 1:1 line, the dashed lines indicate a factor of two difference between observation and prediction.

Figure 5. Endpoint–specific α values from this study (solid circles), compared with those of De Schamphelaere and Janssen (2006) (open circles). Hv ≡ *Hordeum vulgare* root elongation; Le ≡ *Lycopersicon esculentum* shoot elongation; Fc ≡ *Folsomia candida* reproduction; Ef ≡ *Eisenia fetida* reproduction; PN ≡ potential nitrification; MRM ≡ maize residue mineralization; GIR ≡ glucose–induced respiration; Ps ≡ *Pseudokirchneriella subcapitata* growth rate (72 hours); Cv ≡ *Chlorella vulgaris* growth rate (72 hours). Error bars refer to 95% confidence intervals calculated by bootstrapping (endpoints of this study) and 2 × the standard error (endpoints of De Schamphelaere and Janssen, 2006).

Figure 6. Example of variations in Na, Mg, K and Ca concentrations in soil solution with increasing Cu dose. Concentrations of Na, Mg, K and Ca measured in soil solution from Rhydtalog soil following test of *H. vulgare* root elongation, as a function of the measured Cu dose to the soil. Closed circles: Na; open circles: Mg; closed triangles: K; open triangles: Ca. The lines are for guidance.

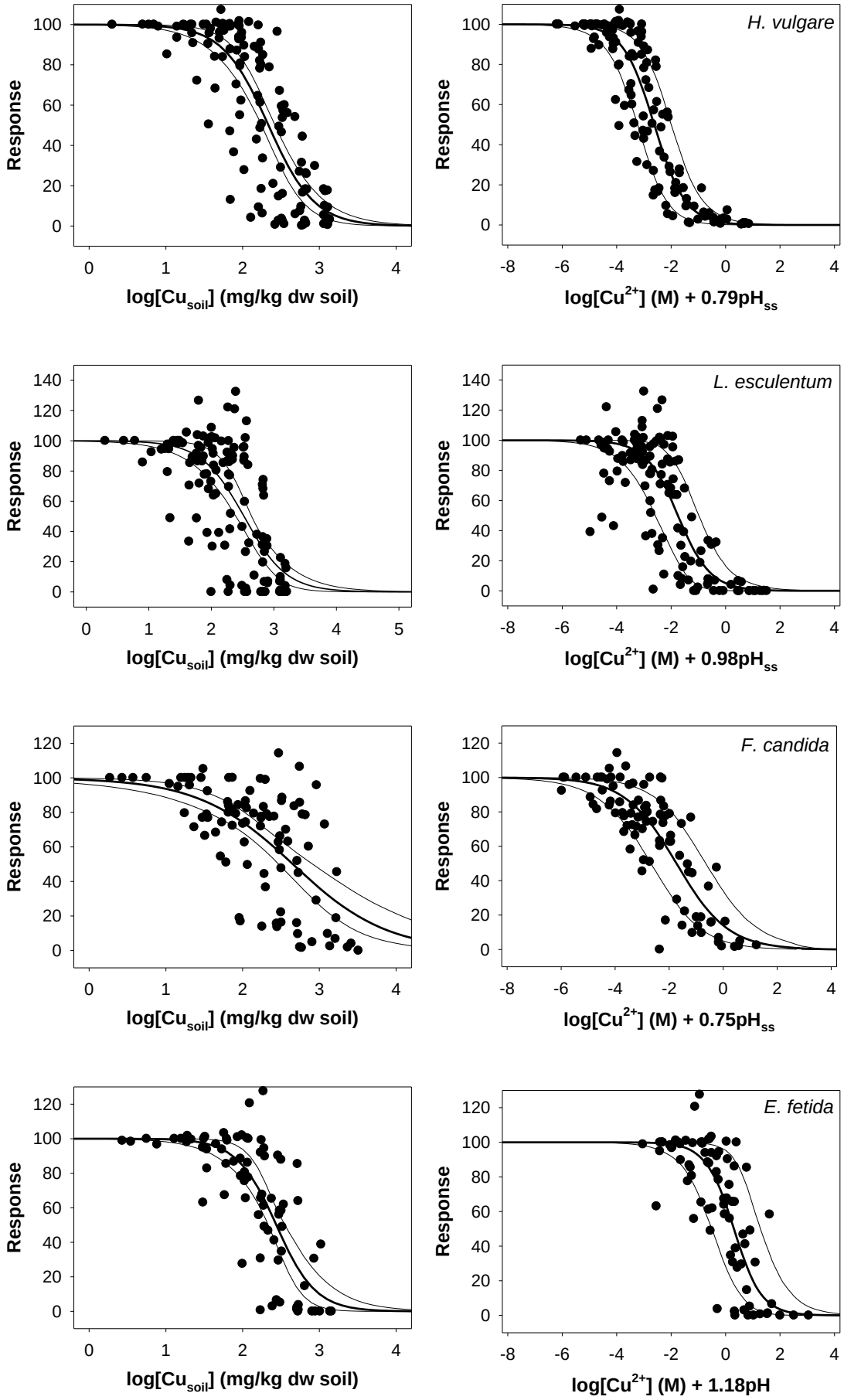
Response
log[Cu$_{soil}$] (mg/kg dw soil)
log[Cu^{2+}] (M) + 0.79pH$_{ss}$
H. vulgare
log[Cu^{2+}] (M) + 0.98pH$_{ss}$
L. esculentum
log[Cu^{2+}] (M) + 0.75pH$_{ss}$
F. candida
log[Cu^{2+}] (M) + 1.18pH
E. fetida

Figure 1.

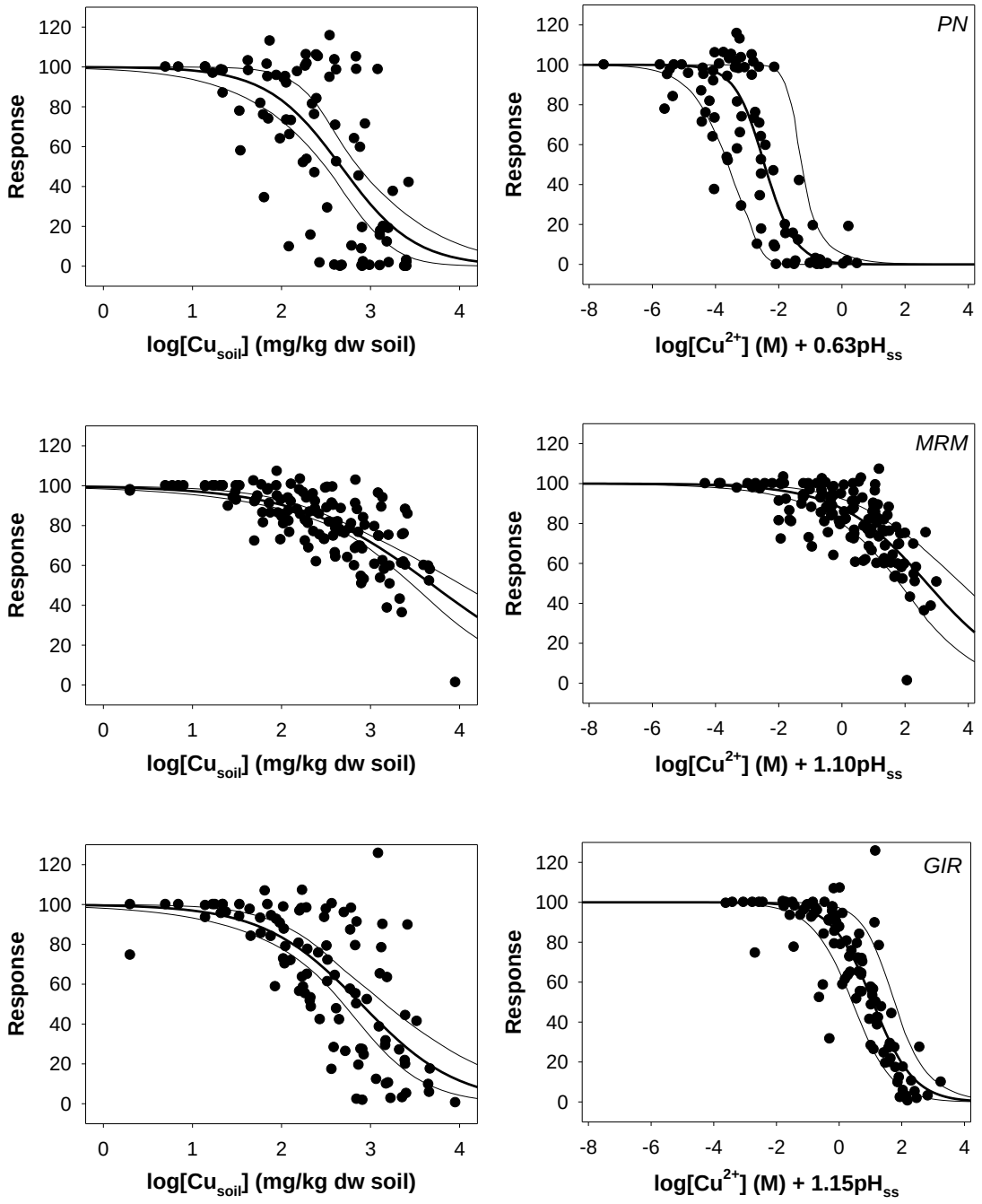
Response
log[Cu$_{soil}$] (mg/kg dw soil)
log[Cu^{2+}] (M) + 0.63pH$_{ss}$
PN
log[Cu^{2+}] (M) + 1.10pH$_{ss}$
MRM
log[Cu^{2+}] (M) + 1.15pH$_{ss}$
GIR

Figure 2.

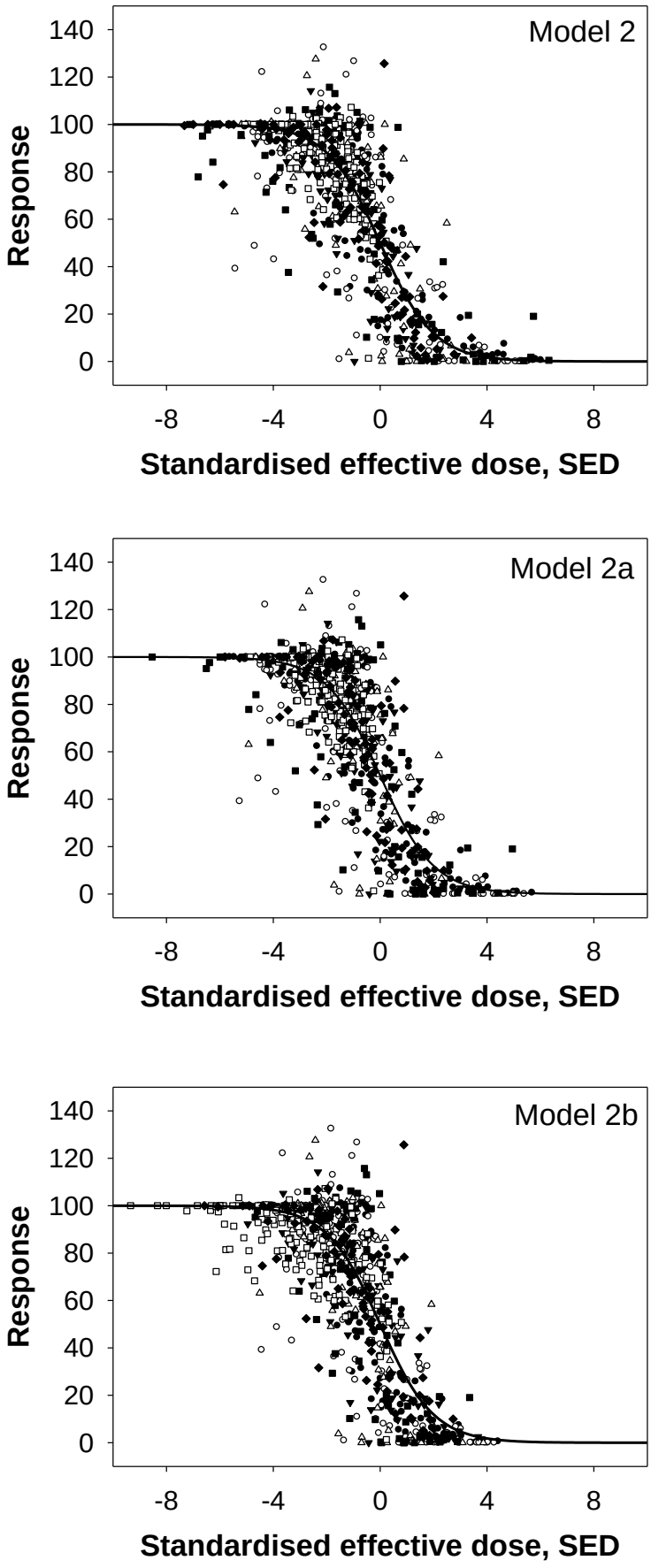
Model 2
Response
140
120
100
80
60
40
20
0
-8
-4
0
4
8
Standardised effective dose, SED
Model 2a
Response
140
120
100
80
60
40
20
0
-8
-4
0
4
8
Standardised effective dose, SED
Model 2b
Response
140
120
100
80
60
40
20
0
-8
-4
0
4
8
Standardised effective dose, SED

Figure 3.

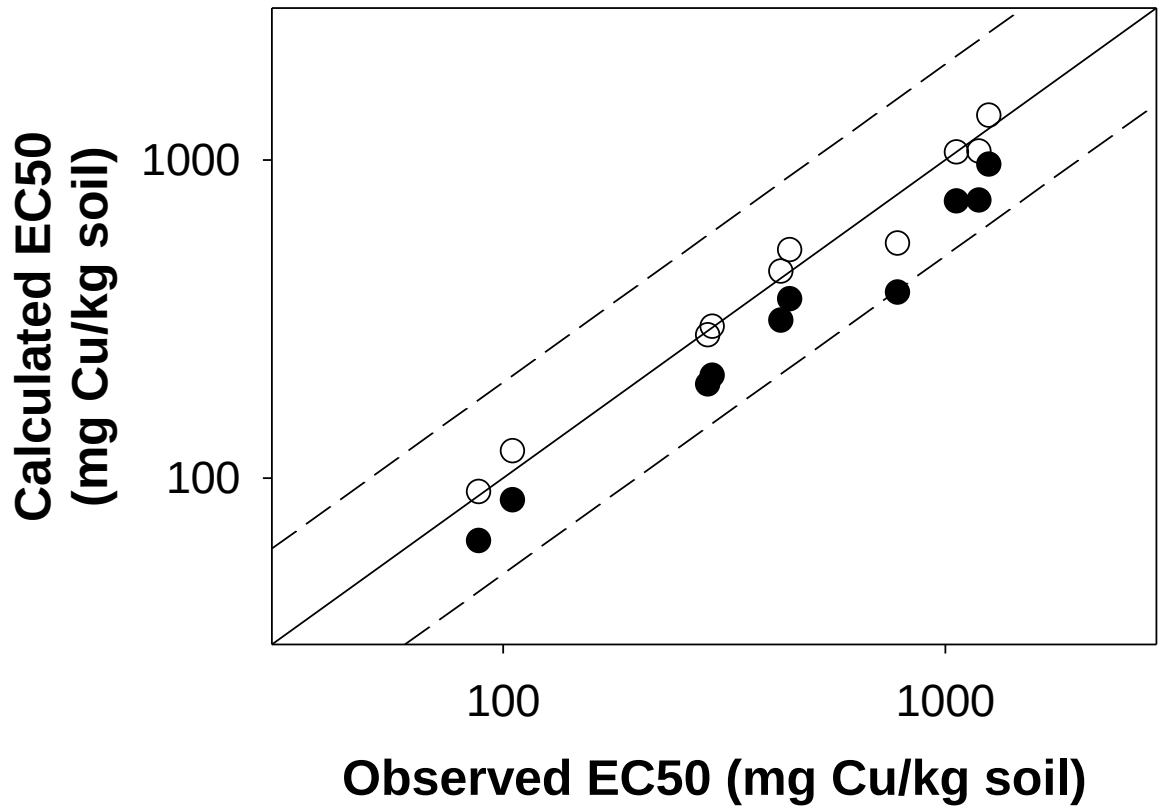
Calculated EC50
(mg Cu/kg soil)
1000
100
100
1000
Observed EC50 (mg Cu/kg soil)

Figure 4.

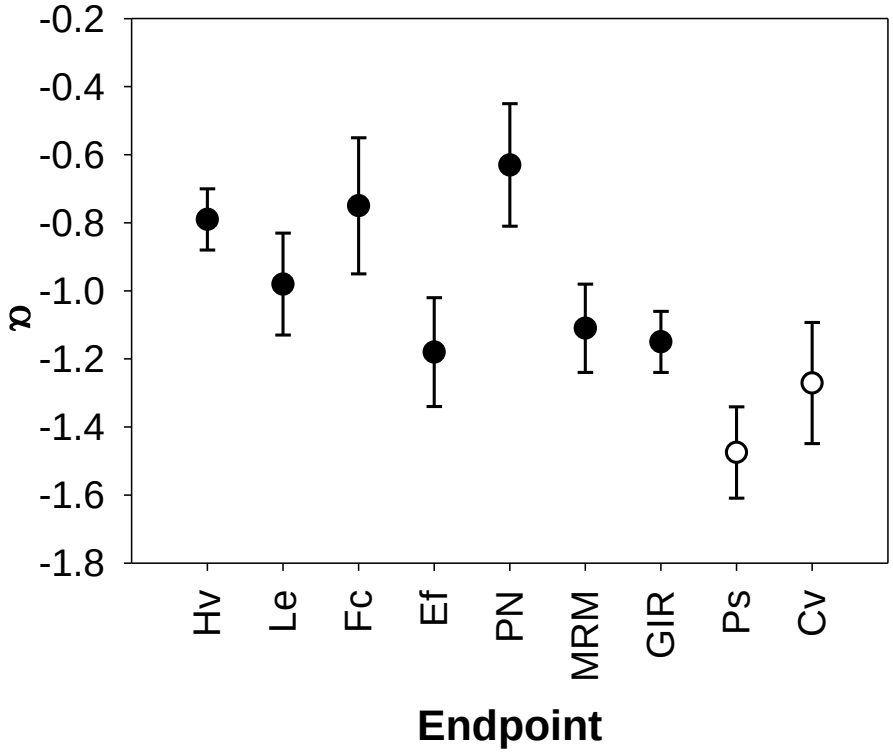

Figure 5.

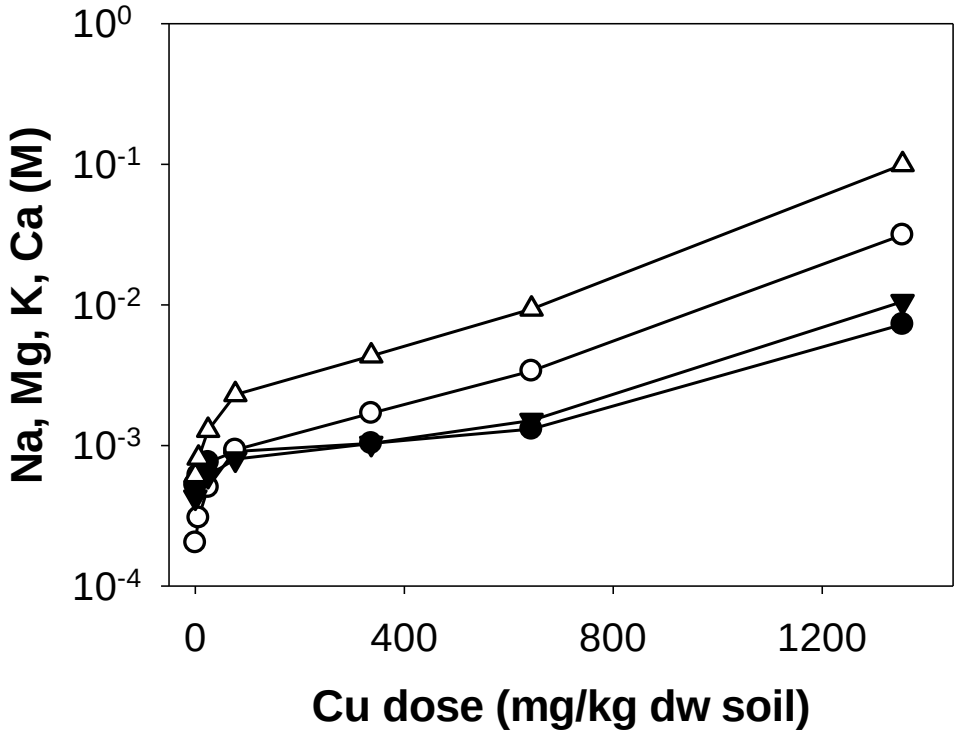
10^0
10^-1
10^-2
10^-3
10^-4
Na, Mg, K, Ca (M)
0
400
800
1200
Cu dose (mg/kg dw soil)

Figure 6.

References

Bongers, M., Rusch, B., Van Gestel, C.A.M., 2004. The effect of counterion and percolation on the toxicity of lead for the springtail *Folsomia candida* in soil. Environmental Toxicology and Chemistry 23, 195–199.

Broos, K., Warne, MStJ., Heemsbergen, D.A., Stevens, D., Barnes, M.B., Correll, R.L., McLaughlin, M.J., 2007. Soil factors controlling the toxicity of copper and zinc to microbial processes in Australian soils. Environmental Toxicology and Chemistry 26, 583–590.

Cheng, T., Allen, H.E., 2001. Prediction of uptake of copper from solution by lettuce (*Lactuca sativa* Romance). Environmental Toxicology and Chemistry 20, 2544–2551.

Criel, P., Lock, K., Van Eeckhout, H., Oorts, K., Smolders, E., Janssen, C.R., 2008. Influence of soil properties on copper toxicity for two soil invertebrates. Environmental Toxicology and Chemistry 27, 1748–1755.

De Schamphelaere, K.A.C., Janssen, C.R., 2006. Bioavailability models for predicting copper toxicity to freshwater green microalgae as a function of water chemistry. Environmental Science and Technology 40, 4514–4522.

De Vos, C.H.R., Schat, H., Vooijs, R., Ernst, W.H.O., 1991. Increased resistance to copper induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. Physiologia Plantarum 82, 523–528.

De Vries, W., Lofts, S., Tipping, E., Meili, M., Groenenberg, J.E., Schutze, G., 2007. Impact of soil properties on critical concentrations of cadmium, lead, copper, zinc, and mercury in soil and soil solution in view of ecotoxicological effects. Reviews of Environmental Contamination and Toxicology 191, 47–89.

Flemming, C.A., Trevors, J.T., 1989. Copper toxicity and chemistry in the environment: a review. Water Air and Soil Pollution 44, 143–158.

Groenenberg, J.E., Römkens, P.F.A.M., Comans, R.N.J., Luster, J., Pampura, T., Shotbolt, L., Tipping, E., de Vries, W., 2010. Transfer functions for solid-solution partitioning of cadmium, copper, nickel, lead and zinc in soils: derivation of relationships for free metal ion activities and validation with independent data. European Journal of Soil Science 61, 58–73.

Kinraide, T.B., Pedler, J.F., Parker, D.R., 2004. Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminium, sodium, and low pH. Plant and Soil 259, 201–208.

Lauren, D.J., McDonald, D.G., 1986. Influence of water hardness, pH, and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Canadian Journal of Fisheries and Aquatic Sciences 43, 1488–1496.

Letelier, M.E., Lepe, A.M., Faundez, M., Salazar, J., Marin, R., Aracena, P., Speisky, H., 2005. Possible mechanisms underlying copper-induced damage in biological membranes leading to cellular toxicity. Chemico-Biological Interactions 151, 71–82.

Lexmond, T.M., 1980. The effect of soil–pH on copper toxicity to forage maize grown under field conditions. Netherlands Journal of Agricultural Science 28, 164–183.

Li, B., Ma, Y., McLaughlin, M.J., Kirby, J.K., Cozens, G., Liu, J., 2010. Influences of soil properties and leaching on copper toxicity to barley root elongation. Environmental Toxicology and Chemistry 29, 835–842.

Lock, K., De Schamphelaere, K.A.C., Becaus, S., Criel, P., Van Eeckhout, H., Janssen, C.R., 2006. Development and validation of an acute biotic ligand model (BLM) predicting cobalt toxicity in soil to the potworm *Enchytraeus albidus.* Soil Biology and Biochemistry 38, 1924–1932.

Lock, K., De Schamphelaere, K.A.C., Becaus, S., Criel, P., Van Eeckhout, H., Janssen, C.R., 2007. Development and validation of a terrestrial biotic ligand model predicting the effect of cobalt on root growth of barley (*Hordeum vulgare*). Environmental Pollution 147, 626–633.

Lofts, S., Spurgeon, D.J., Svendsen, C., Tipping, E., 2004. Deriving soil critical limits for Cu, Zn, Cd, and Pb: A method based on free ion concentrations. Environmental Science and Technology 38, 3623–3631.

Luo, X.S., Li, L.Z., Zhou, D.M., 2008. Effect of cations on copper toxicity to wheat root: Implications for the biotic ligand model. Chemosphere 73, 401–406.

Ma, Y., Lombi, E., Oliver, I.W., Nolan, A.L., McLaughlin, M.J., 2006. Long–term aging of copper added to soils. Environmental Science and Technology 40, 6310–6317.

Ma, F., Huang, H.F., Lin, L.P., Xue, C.H., Li-Ling, J., Chen, L.M., Wang, Y.Q., Li, Q.W., Li, Y.D., 2005. Phylogenetic analysis of Na+/K+ ATPase: Insight into the mechanism for the genesis of multi-isoforms of protein complex. Journal of Biological Systems 13, 299–312.

Mertens, J., Degryse, F., Springael, D., Smolders, E., 2007. Zinc toxicity to nitrification in soil and soilless culture can be predicted with the same biotic ligand model. Environmental Science and Technology 41, 2992–2997.

Oorts, K., Ghesquiere, U., Swinnen, K., Smolders, E., 2006. Soil properties affecting the toxicity of $CuCl_2$ and $NiCl_2$ for soil microbial processes in freshly spiked soils. Environmental Toxicology and Chemistry 25, 836–844.

Oorts, K., Ghesquiere, U., Smolders, E., 2007. Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. Environmental Toxicology and Chemistry 26, 1130–1138.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., Wu, K.B., 2002. The biotic ligand model – a historical overview. Comparative Biochemistry and Physiology C 133, 3–35.

Pressley, T.A., 1992. Phylogenetic conservation of isoform-specific regions within alpha-subunit of Na(+)-K(+)-ATPase. American Journal of Physiology: Cell Physiology 262, C743–C751.

Rooney, C., Zhao, F.J., McGrath, S.P., 2006. Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils. Environmental Toxicology and Chemistry 25, 726–732.

Schwartz, G., 1978. Estimating the dimension of a model. Annals of Statistics 5, 461–464.

Smolders, E., Oorts, K., Van Sprang, P., Schoeters, I., Janssen, C.R., McGrath, S.P., McLaughlin, M.J., 2009. The toxicity of trace metals in soil as affected by soil type and ageing after contamination: using calibrated bioavailability models to set ecological soil standards. Environmental Toxicology and Chemistry 28, 1633–1642.

Spurgeon, D.J., Lofts, S., Hankard, P.K., Toal, M., McLellan, D., Fishwick, S., Svendsen, C., 2006. Effect of pH on metal speciation and resulting metal uptake and toxicity for earthworms. Environmental Toxicology and Chemistry 25, 788–796.

Steenbergen, N.T.T.M., Iaccino, F., De Winkel, M., Reijnders, L., Peijnenburg, W.J.G.M., 2005. Development of a biotic ligand model and a regression model predicting acute copper toxicity to the earthworm *Aporrectodea caliginosa*. Environmental Science and Technology 39, 5694–5702.

Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.J., McGrath, S.P., 2006a. A Terrestrial Biotic Ligand Model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. Environmental Science and Technology 40, 7085–7093.

Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.J., McGrath, S.P., Criel, P., Van Eeckhout, H., Janssen, C.R., Oorts, K., Smolders, E. 2006b. Terrestrial biotic ligand model. 2. Application to Ni and Cu toxicities to plants, invertebrates, and microbes in soil. Environmental Science and Technology. 40, 7094–7100.

Tipping, E., 1994. WHAM - a chemical-equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site electrostatic model of ion-binding by humic substances. Computers and Geosciences 20, 973–1023.

Tipping, E., 1998. Humic Ion Binding Model VI: An improved description of the interactions of protons and metal ions with humic substances. Aquatic Geochemistry 4, 3–48.

Tipping, E., Rieuwerts, J., Pan, G., Ashmore, M.R., Lofts, S., Hill, M.T.R., Farago, M.E., Thornton, I., 2003. The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. Environmental Pollution 125, 213–225.

Warne, MStJ., Heemsbergen, D., Stevens, D., McLaughlin, M., Cozens, G., Whatmuff, M., Broos, K., Barry, G., Bell, M., Nash, D., Pritchard, D., Penney, N., 2008. Modeling the toxicity of copper and zinc salts to wheat in 14 soils. Environmental Toxicology and Chemistry 27, 786–792.

Zhao, F.J., Rooney CP, Zhang H, McGrath, SP. 2006. Comparison of soil solution speciation and DGT measurement as an indicator of copper bioavailability to plants. Environmental Toxicology & Chemistry 25, 733–742.