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Limitations of experiments performed in artificially made OECD standard soils for predicting cadmium, lead and zinc toxicity towards organisms living in natural soils

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Abstract

Development of comparative toxicity potentials of cationic metals in soils for applications in hazard ranking and toxic impact assessment is currently jeopardized by the availability of experimental effect data. To compensate for this deficiency, data retrieved from experiments carried out in standardized artificial soils, like OECD soils, could potentially be tapped as a source of effect data. It is, however, unknown whether such data are applicable to natural soils where the variability in pore water concentrations of dissolved base cations is large, and where mass transfer limitations of metal uptake can occur. Here, free ion activity models (FIAM) and empirical regression models (ERM, with pH as a predictor) were derived from total metal EC50 values (concentration with effects in 50% of individuals) using speciation for experiments performed in artificial OECD soils measuring ecotoxicological endpoints for terrestrial earthworms, potworms, and springtails. The models were validated by predicting total metal based EC50 values using backward speciation employing an independent set of natural soils with missing information about ionic composition of pore water, as retrieved from a literature review. ERMs performed better than FIAMs. Pearson's r for $\log_{10}$-transformed total metal based EC50s values (ERM) ranged from 0.25 to 0.74, suggesting a general correlation between predicted and measured values. Yet, root-mean-square-error (RMSE) ranged from 0.16 to 0.87 and was either smaller or comparable with the variability of

measured EC50 values, suggesting modest performance. This modest performance was mainly due to the omission of pore water concentrations of base cations during model development and their validation, as verified by comparisons with predictions of published terrestrial biotic ligand models. Thus, the usefulness of data from artificial OECD soils for global-scale assessment of terrestrial ecotoxic impacts of Cd, Pb and Zn in soils is limited due to relatively small variability of pore water concentrations of dissolved base cations in OECD soils, preventing their inclusion in development of predictive models. Our findings stress the importance of considering differences in ionic composition of soil pore water when characterizing terrestrial ecotoxicity of cationic metals in natural soils.

Keywords: biotic ligand; free ion; life cycle assessment; metals; soils

1. Introduction

Addressing liquid-phase speciation in calculation of comparative toxicity potentials (CTP) for application in hazard ranking and toxic impact assessment requires that both the bioavailability factor and the effect factor used in the CTP calculation must be based on immediately bioavailable toxic metal forms (Gandhi et al. 2010; Owsianiak et al. 2013; Dong et al. 2014). The bioavailability factor used in CTP calculations is expressed as the fraction of metal present in the directly bioavailable, toxic forms, relative to the reactive metal concentration (Owsianiak et al. 2013). The effect factor indicates the average toxic potency of the directly bioavailable, toxic forms of a metal. This effect factor is derived from free ion based HC50 values, the hazardous concentration of toxic metal forms affecting 50% of the species, calculated as a geometric mean of EC50 values for individual species (i.e. the concentration with (lethal) effects in 50% of the individuals of a species). As EC50s are based on either free ion or truly dissolved metal concentrations (i.e. including free ions and inorganic complexes) they can be derived using either terrestrial biotic ligand models (TBLM), empirical regression models (ERM), or free ion activity models (FIAM) (Owsianiak et al. 2013; Qiu et al. 2013). ERMs can be considered as an intermediate approach between relatively simple FIAMs, which assume that the ecotoxic response is proportional to metal free ion activity in the pore water, and more complex TBLMs, which assume that the ecotoxic response is proportional to the amount of metal ions bound to biotic ligand as influenced by protons and base cations. Protons and base cations compete with toxic metal ions for binding to the biotic ligand of the exposed organism.

Currently, the development of free ion based EC50 values for cationic metals in soils is constrained by the availability of terrestrial effect data of sufficient quality needed to derive them. The major limitation of reported effect data is incomplete information about ionic composition of soil pore water in the tested natural soils, which influences both speciation pattern of a metal and the ecotoxic response through competitive binding of protons and sometimes base cations to biotic ligand(s) (Steenbergen et al. 2005; Thakali et al. 2006a,b; Voigt et al. 2006). Incomplete information about soil properties has led to disregarding speciation in the effect factor of Zn, resulting in an underestimation of the CTP (Plouffe et al. 2015a, 2016). It is thus important to find alternative sources of data, which can be used to derive models predicting EC50 values of metals in soils based on directly available, toxic metals forms.

Ecotoxicological effect data measured in artificial soils, like OECD soils, could potentially be tapped as a source of data for calculation of free ion based HC50 values as the composition of the OECD soils is known and pore water compositions can be estimated. Indeed, in the ECOTOX database (U.S. Environmental Protection Agency 2012) the majority of ecotoxicity tests for common cationic metals with the terrestrial earthworm *Eisenia fetida* were conducted in artificial soils (59, 95 and 86% of all experiments with *E. fetida* for Cd, Pb and Zn, respectively). Some metals have data from artificial soils only (e.g. Au, Ti). It is therefore of interest to evaluate the applicability of models built on effect data measured in artificial OECD soils for predicting metal ecotoxicity in natural soils while considering variability in properties of natural soils.

It is hypothesized that the difference in ionic composition of the water phase between artificial OECD soils and natural soils will limit the applicability of effect data from experiments carried out in artificial OECD soils. Although average pore water concentrations of base cations in artificial OECD soils (Lock et al. 2006) and natural soils (Owsianiak et al. 2013) are usually within the same order of magnitude (with the exception of Ca^{2+} concentration which on average is by one order of magnitude higher in natural soils), the variability in pore water concentration of base cations is much higher in natural soils, where differences by up to three, (Na^{+}, K^{+}), five (Ca^{2+}) and six (Ca^{2+}) orders of magnitude are observed (Owsianiak et al. 2013). An increase in concentrations of dissolved Mg^{2+} by one order of magnitude decreases toxicity to various terrestrial organisms towards Ni^{2+} by a factor of five (Owsianiak et al. 2013). Thus, the applicability of models based on effect data measured in artificial OECD soils is expected to depend on: (i) ionic composition of the artificial OECD soils used to derive the predictive models, and (ii) the ionic composition of the natural soil(s) where the model is employed for prediction of metal's ecotoxicity. Ionic composition of pore water is rarely measured in ecotoxicity experiments and is not reported in soil databases like ISRIC-WISE3 or the Harmonized World Soil Database (HWSD) (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009), and is not always possible to calculate (e.g. HWSD does not provide information about exchangeable base cations, which also span a wide range in natural soils). Thus, it is of interest to estimate the implications of the limited information about ionic composition of soil pore water on the performance of models developed based on effect data from experiments carried out in artificial OECD soils. Although there is some variability in properties of artificial OECD soils, which can influence sorption and resulting ecotoxicity, the extent of this variability is smaller compared to the variability in properties of natural soils (Crommentuijn et al. 1997; Bielská et al. 2012, 2017; Hofman et al. 2014; Vašíčková et al. 2015). Geographic variability in properties of natural soils must be considered when computing CTP of metals at a global scale (Plouffe et al. 2016; Owsianiak et al. 2013).

The aim of this study was to evaluate the applicability of free ion based models (FIAM and ERM) derived from effect data measured in artificial OECD soils for predicting ecotoxicity of cationic metals in natural soils at the level of total metal based EC50s. For this purpose, the empirical data from a literature review based solely on data from experiments performed in artificial OECD soils were collected and subjected to speciation modelling to develop FIAMs and ERMs separately for various species of earthworms, potworms, and springtails for acute and chronic endpoints, like mortality, growth, and reproduction. Next, using backward speciation, the models' performance for prediction of total metal based EC50 values in natural soils was tested. To quantify the influence of missing data about pore water concentration of dissolved based cations both in models' development and validation, comparison was made with prediction of published terrestrial biotic ligand models using pore water concentrations of base cations being in average, lower, and higher range of values expected for global soils.

2. Methods

The study involved collection and selection of data from OECD soils based on defined set of criteria, as presented in Fig. 1. Then speciation calculations for the OECD soils to derive FIAMs and ERMs were conducted. Finally, backward speciation calculations to total metal content were done on a data set representing natural soils selected, applying the same criteria as for selection of the data in OECD soils, and the model predictions of ecotoxicity in these soils were evaluated.

2.1. Data collection and selection criteria

Data on metal ecotoxicity were collected from peer-reviewed scientific reports available until March 2015 identified through searching the ISI Web of Science, v. 5.17 (Thomson Reuters, New York, NY), using a combination of keywords: (i) “toxicity”; and (either) (ii) “soil”, or “terrestrial”; and (either) (iii) “Al”, “Ba”, “Be”, “Cd”, “Co”, “Cr”, “Cs”, “Fe”, “Mn”, “Pb”, “Sr”, “Zn”, “aluminum”, “barium”, “beryllium”, “cadmium”, “cobalt”, “chromium”, “cesium”, “iron”, “manganese”, “lead”, “strontium”, or “zinc”; and (either) (iv) “EC50”, “LC50”. For example, one of the used keywords combination was: “toxicity” and “soil” and “Zn” and “EC50”. A complementary search was conducted in ISI in order to collect publications citing references retrieved in the previous step, and those which were cited in the collected publications, but were not found through the initial search. The two latter steps were iterated until no new data were found. Although the ecotoxicity effect data for Cu and Ni is relatively abundant, effect factors of these two metals were already calculated using terrestrial biotic ligand models (Owsianiak et al. 2013). Cu and Ni are thus not considered as priority metals for calculation of effect factors underlying CTP.

2.1.1. Organisms and ecotoxicity endpoints

In the study three groups of soil invertebrates were included: (i) earthworms, (ii) potworms, and (iii) springtails. In total 18 species were considered. Details of the organisms included are presented in Table 1.

The following criteria were applied when including ecotoxicity data: (i) ecotoxic endpoint was reported; (ii) ecotoxicity test was done only using single metal; and (iii) duration of the exposure was reported. In summary, the following endpoints were considered: (a) growth (including such endpoints as fresh weight, dry weight or growth rate), (b) reproduction (including such endpoints as juvenile production, number of juveniles, offspring production, cocoon production, egg deposition), (c) population size (including population increase endpoint), (d) sexual development and (e) mortality. Behavioral and biomarker endpoints, such as neutral-red retention assay, were excluded.

2.1.2. Metals

Only soils spiked with readily soluble metal salts were considered. Only the elements for which the number of independent ecotoxicity experiments was >10 per group of organisms (i.e. earthworms, potworms and springtails), separately for artificial OECD soils and for natural soils, were included. In practice, only Cd, Pb, and Zn met this criterion.

2.1.3. Artificial OECD soils and natural soils

Experiments performed in artificial OECD soils must report the soil pH and soil organic carbon (SOC) or soil organic matter (SOM) content. The same criteria applied for natural soils. For natural soils, various agricultural, grassland and forest soils, as well as commercial soils (like LUFA 2.2 standard soil and Broughton Kettering loam) were included. To the extent possible, data on clay content, cation exchange capacity (CEC), dissolved organic carbon (DOC), electric conductivity of the soil (EC), and pore water concentration of base cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) were included. However, the availability of this data was low (<3% of all data).

2.2. Harmonization of collected data

As only soils (either artificial or natural) spiked with water-soluble salts of Cd, Pb or Zn (usually nitrates or chlorides) were considered, many studies assumed their contents in soils to be equal to the nominal concentration (proportional to the applied weighted portion of particular metal salt), disregarding background metal concentration. Several studies reported not only nominal, but also measured concentration of metal (the concentrations were measured using flame atomic absorption spectrometry, while the soil samples were obtained by soil digestion in hot acid being a combination of different volumes of HCl, HNO_3, and (sometimes) deionized water). If both nominal and measured values were reported, the measured values were used. It was assumed, that all of the methods for determination of total metal concentration in the soil are equivalent.

The EC50 values corresponding to total metal concentration were normalized to mg kg^{-1} dry soil values. An empirical regression developed by Azevedo et al. (2013) was applied to convert soil pH values measured in KCl- or $CaCl_2$-extracts to values corresponding to measurements in H_2O. When the pH measurement method was not mentioned in the study, it was assumed that all of the measurements made before 2005 were conducted in H_2O extracts. For all the reports published after 2005, it was assumed that pH measurements were made in $CaCl_2$-extracts. These assumptions are based on two OECD guidelines published in 2004 (OECD 220, 2004; OECD 222, 2004), which obligated scientist (using OECD artificial soil) to use 1 M KCl or 0.01 M $CaCl_2$ solution during pH measurements, and on the fact that after 2005, a majority of the studies (also including tests in natural soils) measured pH with the use of 0.01 M $CaCl_2$ (i.e. after 2005 pH_{CaCl2} values were reported in 87 of 151 (57.6%) of available data points (considering both artificial matrices and soils) mentioning at least one of the pH measurement methods). A ratio of SOM (soil organic matter) to SOC (soil organic carbon) equal to 1.78 was applied, and total soil carbon was assumed to contain 75% of SOC (Batjes, 1996). The values of CEC were normalized to cmolc kg^{-1} dry soil.

The data points with values of EC50, pH, CEC or DOC reported as below or above a certain value were excluded. Moreover, if any confidence intervals of the values were reported without mean value, the median value was used. If some data reported changes in pH, CEC or DOC during the experiments, the arithmetic mean value of all reported values for particular data point was used.

2.3. Development of free ion activity models (FIAM)

FIAMs were developed per metal, organism, ecotoxicological endpoint and duration of exposure. First, free ion based EC50 was calculated separately for each effect data point from total metal based EC50 using speciation calculations. As FIAM assumes that ecotoxic response is proportional to free ion activity of a metal in pore water, it was expressed as the geometric mean of all endpoint-specific free ion based EC50s values (Eq 1); that is, a fixed activity of free metal ion in pore water causes a toxic effect.

$$\mathrm{EC50}_{\{Me^{2+}\}} = \sqrt[i]{\prod \mathrm{EC50}_{i,\{Me^{2+}\}}} \qquad \text{Eq. 1.}$$

where $\mathrm{EC50}_{\{Me^{2+}\}}$ is the average (geometric mean) concentration with effects in 50% of the individuals of a species corresponding; $\mathrm{EC50}_{i,\{\mathrm{Me}^{2+}\}}$ is the free ion based EC50 value of a metal Me^{2+}; and *i* is the number of included free ion based EC50 values used to derive respective FIAM.

In total, 29 FIAMs were developed. Note, that expressing the FIAM as geometric mean, although common in toxic impact assessment and sufficient for calculation of free ion based HC50 values (Gandhi et al. 2011a, 2011b; Dong et al. 2014) does not allow for computing response at other levels of affected fraction of organisms (e.g. EC5, EC10) as dose-response parameter is not known (Thakali et al. 2006a). Derivation of full FIAM with the dose-response parameter was outside the scope of the study.

2.4. Development of empirical regression models (ERM)

Empirical regression models were developed as alternative to FIAMs to take into account the influence of protons on metal ecotoxicity. Soil pH was included as independent variable, as protons are important predictors of (free ion) ecotoxicity of cationic metals (Erickson et al. 1996; Meyer et al. 1999; Lofts et al. 2004; Ardestani et al. 2013). The inclusion of the pH in the regression for free ion based EC50 includes both the expected increase in ecotoxicity due to higher free activity of toxic cations at low pH, and a decrease in ecotoxicity due to competition from protons for binding to the biotic ligand on the organism. As OECD soils are standardized and almost none report mentioned the measured base cations concentration, the variability in dissolved concentration of base cations in artificial OECD soils was not included in model development. As for FIAMs, free ion based EC50 values (derived from total metal EC50 values by means of speciation calculations) were used. Free ion EC50 values corresponding to the same pH were averaged (geometric mean) before entering the regression. The EC50 values were $\log_{10}$-transformed as it improved normality of their distribution as verified using Kolmogorov-Smirnov test (Eq. 2). Regressions were developed if the number of free ion based EC50 values with different pH values was ≥ 5. In total, nine ERMs were developed.

$$\log_{10} \mathrm{EC50}_{\{Me^{2+}\}} = \mathrm{a} \times \mathrm{pH} + \mathrm{b} \qquad \text{Eq. 2.}$$

where *a* and *b* are regression coefficients. In addition to the regression parameters, the following parameters were calculated: R^2 (coefficient of determination), se (residual standard error of regression) and *p* value (regression probability level).

As for FIAMs, ERMs were developed per metal, organism, endpoint, and duration of exposure. ERM allows computing free ion based EC50 specific to pore water pH.

2.5. Evaluation of model performance

FIAMs and ERMs derived using ecotoxicity data measured in artificial OECD soils were validated by computing respective, total metal based EC50 in the natural soil and comparing with measured values. Evaluation of applicability of FIAMs and ERMs for predicting metal ecotoxicity in natural soils was quantified using root mean square error (RMSE), bias (that is, mean error) and the Pearson's Product moment correlation, PPMC (also known as Pearson's *r*). RMSE quantifies the difference between predicted and measured values, bias indicates whether the model tends to under- or over-estimate the measured data, while PPMC quantifies the correlation between predicted and measured values. All three parameters are often used in characterizing performance of environmental models (Groenenberg et al. 2010; Bennett et al. 2013).

2.6. Speciation and backward speciation

Free ion based underlying FIAMs and ERMs were derived from total metal based EC50 values in artificial OECD soils by means of whole soil metal speciation using WHAM7 (Centre for Ecology & Hydrology, United Kingdom), following the approach of (Thakali et al. 2006a) as explained in Appendix A1. Input parameters for the speciation are presented in Appendix A2 (Tables A1-A3).

Backward speciation was carried out in WHAM7 using free ion based EC50 value predicted using either FIAM or ERM and properties of natural soils as input. In the absence of measured concentrations of dissolved base cations, they were either retrieved from literature, calculated, or assumed. For LUFA 2.2 standard soil (LUFA Speyer, Germany), dissolved concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+} were made equal to pore water concentration values measured by Lock et al. (2006). For the Kettering loam soil (Boughton Loam Ltd, United Kingdom), they were calculated following the Gaines-Thomas convention (Gaines and Thomas, 1953; Vulava et al. 2000) for modeling cation exchange, using available exchangeable ion concentrations (equal to values measured by Lambkin et al. (2011) and electrical conductivity of soil pore water (equal to 0.28 mS/cm as measured in Page et al. (2014)), following the approach of Owsianiak et al. (2013). For other soils, the dissolved concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} ions were assumed equal to median concentrations (Na^+: 1.80E-03 [M], K^+: 6.60E-04 [M], Mg^{2+}: 3.80E-04 [M], Ca^{2+}: 7.40E-04 [M]) calculated across 760 global, non-saline (ionic strength of soil pore water below 0.5 mol/L) soils (Owsianiak et al. 2013). To test the implications of this assumption, backward speciation was also done for base cation concentrations corresponding to 2.5th and 97.5th percentile of the values calculated for a global set of 760 soils by Owsianiak et al. (2013). Details of the backward speciation are presented in Appendix A1. The input data for backward speciation are presented in Appendix A2 (Tables A4-A6).

3. Results

A total number of 623 ecotoxicity data points retrieved from 85 published papers was collected. The overview of the collected data is presented in Table 1. Nearly two-third of the data points are derived from ecotoxicity tests conducted in artificial OECD soils, like the OECD standard soil and its variants. Total metal based EC50 values measured in either artificial OECD soils or in natural soils ranged over three orders of magnitude across all organisms (Table 1). However, the average (geometric mean) variability of total metal based EC50 values (for the same pH) was usually within two orders of magnitude for individual endpoints. For four endpoints (considering all three metals), the total metal based EC50s increased (i.e. ecotoxicity decreased) by up to one order of magnitude with increasing pH by three units (see Appendix A3, Fig. A1). For some metals and some organisms, however, no apparent change in ecotoxicity with pH was observed. Generally, based on average (geometric mean) total metal based EC50 values, the most toxic metal in artificial OECD soils was Cd, followed by Zn and Pb. The same metal ecotoxicity ranking as in artificial OECD soils was observed for natural soils. However, apart from Cd, the variability in total metal based EC50 measured in natural soils was smaller (by one order of magnitude) as compared to artificial OECD soils; in the OECD soils the lowest EC50 values were generally one order of magnitude higher as compared to natural soils for a comparable number of data points (Table 1).

Table 2 presents all FIAMs derived from total metal based EC50 values measured in artificial OECD soils. Ranking of the three metals changes when EC50 values are based on free ions, with the most toxic metal being Pb, followed by Cd and Zn. Across organisms, free ion based EC50 varied by four (Cd and Zn) and six (Pb) orders of magnitude. Per organism and individual endpoint, the average (geometric mean for the same pH) variability was within two orders of magnitude, which is similar to variability in total metal EC50. For majority of metals and endpoints, the free ion based EC50 decreased by up to 1.5 orders of magnitude with increasing pH by 3 units (see Appendix A3, Fig. A2). With respect to artificial OECD soils, geometric coefficients of variation for free ion based EC50 values were larger than the respective geometric coefficients of variation for the total metal based EC50 values (Table 2), suggesting that just the free ion activity, as predicted using FIAM, is not a sufficient descriptor of metal exposure in artificial OECD soils.

Estimation of total metal based EC50 values in natural soils using FIAMs developed in artificial OECD soils shows that predicted values are within two orders of magnitude around measured, total metal based EC50 value (Fig. 2a-c). Statistical details of the evaluation of FIAMs are presented in Appendix A4, Tables A7-A9. RMSE values (for $\log_{10}$-transformed data) were relatively high (close to, but below one). Across all individual endpoints, the best performance (RMSE lower or equal to 0.45 and PPMC greater than 0.9) was observed for Cd, *F. candida*, growth 28-d endpoint. RMSE values were always above 0.45 for either acute or chronic endpoints, suggesting small, if any, decrease in FIAM performance when either all acute or all chronic data are pooled together. Pooling the data increased the number of data points, increasing precision, which outweighed decrease in performance due to combining various endpoints. Biases were either positive or negative depending on the organisms and metal, and ranged from -1.0 to +1.3. The highest overestimation of ecotoxicity was observed for data points at low pH (Fig. 2a-c). There was observed a clear relationship between squared errors and the pH (the squared errors increase when pH decreases) (see Appendix A3, Fig. A3).

Table 2 shows that ERM regression coefficient *a* for the independent variable pH is negative confirming decreasing free ion based ecotoxicity with decreasing pH value (see Appendix A3, Fig. A2). The performance of ERMs for prediction of total metal based EC50 values in natural soils is shown in Figure 2d-e. Statistical details of the evaluation of ERMs are presented in Appendix A4, Tables A7-A9. For Cd, the best performance of ERM (RMSE lower than 0.5 and bias in the range of −0.5 to +0.5) was observed for *F. candida*. For Zn, the best performance was observed for reproduction 28-d endpoint (*F. candida*) (RMSE equal to 0.47, bias equal to 0.22). Total metal based EC50 values derived from ERMs were generally within two orders of magnitude, which is similar to the performance of FIAMs. Despite smaller number of data points, both RMSE and PPMC values for ERMs were comparable to those of FIAMs, suggesting improved performance when ERMs are used compared to FIAMs. This improvement is apparent in low pH soils (pH < 5), where the ERMs, unlike FIAMs, do not consistently overestimate metal ecotoxicity.

4. Discussion

4.1. The influence of soil pH

The observed variability (per individual endpoint) of free ion based EC50 values (within two orders of magnitude) is smaller than variability reported by Christiansen et al. (2011), who observed that free ion based EC50 values of Cu^{2+} ranged from 0.01 and 16 μg/L) for both acute and chronic experiments performed with freshwater crustacean *D. magna*. However, the authors indicated that the free ion concentration represents the toxic forms of Cu better than the total Cu and attributing the observed variability to experimental uncertainty, uncertainties or errors in the applied speciation modelling, and of the toxicity of less dominant Cu species. In our study on artificial OECD soils, geometric coefficients of variation for free ion based EC50 values were larger than the respective geometric coefficients of variation for the total metal based EC50 values (Table 2), suggesting that just the free ion activity, as predicted using FIAM, is not a sufficient descriptor of metal exposure in artificial OECD soils. The observed lack of change in total metal EC50s with various pH values (for various endpoints) can be explained by two competing mechanisms: increasing concentration of free ions with decreasing pH resulting in increasing ecotoxicity, and protective effect of protons competing with toxic free ions for binding to biotic ligand of the organism (Lofts et al. 2004). This is consistent with literature findings (e.g. Li et al. (2008) showing that decrease in pH from 7.1 to 5.5 resulted in the increase in free ion based LC50 value for *E. fetida*), and is in agreement with predictions of terrestrial biotic ligand models (Ardestani et al. 2013), and is consistent with our observations showing that ERMs taking into account the influence of protons generally perform better than FIAMs.

4.2. Explaining modest performance

It was shown, that the error of prediction of total metal based EC50 values of the three cationic metals in natural soils using free ion based models (FIAM or ERM) developed using effect data measured in artificial OECD soils is either below (in most cases within one to one point four orders of magnitude), or comparable (within two orders of magnitude) with the variability of measured EC50 values, which is around two orders of magnitude for each organism. Thus, the usefulness of such effect data for prediction of metal ecotoxicity in natural soils is modest. This does not mean, however, that there is lack of correspondence between artificial OECD soils and natural soils in terms of metal ecotoxicity. Rather, it means that uncertainties associated with speciation and backward speciation (up to one order of magnitude in prediction of free ion concentration (Groenenberg et al. 2010), combined with limitation of the soil data set (lack of measured pore water concentrations of base cations), can result in the limited applicability. As the direction of bias is not systematic, supply limitations due to likely smaller effective diffusion coefficients of a metal in soil and retardation of a metal in the soil is either not important or are less important for the predictions that inclusion of proton- or base cation-organism interactions.

The pore water concentrations of dissolved base cations can influence both speciation pattern of a metal in the soil and its ecotoxicity through cation-organism interactions. Plouffe et al. (2015b) showed that WHAM-predicted bioavailable fraction of Zn (including free ion and inorganic complexes) had uncertainty of two orders of magnitude, when pore water concentrations of dissolved base cations were estimated from CEC using just soil density. However, pore water concentrations of base cations vary more (from three to six orders of magnitude) than CEC does (two orders of magnitude) (Owsianiak et al. 2013). To show whether the variability in concentration of base cations can explain the modest performance of the FIAMs and ERMs, terrestrial biotic ligand models (TBLMs) developed for *E. fetida* (Li et al. 2008) and *F. candida* (Ardestani et al. 2013) were used to predict free ion based and total metal based EC50s of Cd in natural soils employing concentration of dissolved base cations equal to either median, 2.5th , or 95th percentile values calculated for 760 soils from around the World (Owsianiak et al. 2013). The average free ion based EC50 values (calculated as arithmetic mean across different values of pH) of Cd varied by 1.8 and 2.2 orders of magnitude for *F. candida* and *E. fetida*, respectively (Fig. 3a). However, the variability was smaller for *F. candida* in lower values of pH (pH<6, data not shown). The variability in total metal based EC50s was smaller, being from 0.5 and 0.9 orders of magnitude for *F. candida* and *E. fetida*, respectively (Fig. 3b). Thakali et al. (2006b) compared the performance of FIAM and TBLM for prediction of Cu and Ni toxicity to *E. fetida* (cocoon production) and *F. candida* (juvenile production) and observed better model performance (lower values of RMSE, higher values of R^2) in the case of TBLM (which considered the protective effect of H^+, Ca^{2+} and Mg^{2+}). Other studies indicated either significant (Li et al. 2008) or insignificant (Ardestani et al. 2013) effect of protons and base cations on TBLM performance with respect to Cd toxicity towards soil invertebrates. Moreover, no TBLMs are currently developed for Zn and Pb with respect to soil invertebrates. However, base cations are relevant for metal ecotoxicity for many of the organisms included in this study (Ardestani et al. 2014). Therefore, it could be expected that the performance of ERMs would improve, if the effects of cations would be included in development and application of ERMs.

4.3. Potential influence of metal supply limitations

This study was focused on the influence of ionic composition of pore water, but due to large variability in properties of natural soils, metal supply rate to an organism can also be either lower or higher in natural soils as compared to the metal supply rate in an artificial OECD soils. For example, the diffusion coefficient of a metal in the water phase of artificial OECD soils (with 20% clay content) is nearly twice as high as compared to a natural soil with higher (45%) clay content when at 80% water saturation (which is a typical saturation percentage used in ecotoxicity experiments). This difference increases to a factor of six, if soils are less saturated (50% water content) (Olesen et al. 2001; Moldrup et al. 2007). Clay and organic carbon contents in natural soils vary from 1 to 82% and from 0 to 38%, respectively (as reported for a subset of 760 natural soil profiles from the ISRIC-WISE3 soil database (Batjes, 2009), while OECD soils typically have fixed clay and organic carbon content (Owsianiak et al. 2013). Further, in addition to differences in effective diffusivity of a metal, metal supply to an organism is also influenced by sorption to solid soil constituents, which can also be either smaller or larger in natural soils as compared to OECD artificial soils (e.g. solid/liquid partition coefficient (K_d) of Cd varied by one order of magnitude in artificial OECD soils, while it varied by up to four orders in natural soils) (Bielská et al. 2017). Owsianiak et al. (2014) already showed that metal absorption efficiency by earthworms in soils contaminated with metals from various anthropogenic sources was influenced by the rate of metal supply to the membrane. Supply limitations of metal uptake by an organisms due to sorption and/or low effective diffusivity, if occurring, violate the fundamental assumption of all free ion based ecotoxicity models (Campbell, 1995). As the direction of bias was not systematic in our study, however, supply limitations due to likely smaller effective diffusion coefficients of a metal in soil and retardation of a metal in the soil is either not important or are less important for the predictions that inclusion of proton- or base cation-organism interactions. Supply limitations are, however, thought to be more important in long-term aged soils (Owsianiak et al. 2015), in which case the applicability of models developed in spiked non-aged OECD soils will be challenged further.

Conclusions

This study showed that the applicability of effect data from experiments carried out in artificial OECD soils for prediction of ecotoxicity of Cd, Pb, and Zn in natural soils is limited due to missing information about pore water concentration of base cations in the OECD soils, preventing their inclusion in development of predictive models. This finding has two implications for impact assessment of metals on terrestrial ecosystems.

First, computing comparative toxicity potentials (CTP) using HC50 values derived using effect data retrieved from experiments carried out in OECD soils can either over- or under-estimate the CTP by up to ca. 1 order of magnitude. The extent of this over- or under-estimation will depend both on the spatial scale of an impact assessment where the CTP is employed and geographic variability of pore water concentrations of influential base cations in the soil(s) being assessed. The error will be larger if such a hypothetical OECD-based HC50 is used for assessment in soils where pore water concentrations are consistently different from “average” concentrations in OECD soils, e.g. in agricultural soils limed with Ca- and Mg-rich materials. The error is expected to be smaller in cases where deposition of a metal occurs on large areas, like airborne emissions impacting wide range of natural soils, where average pore water concentrations of base cations could be closer to average concentrations in OECD soils (de Caritat et al. 1997).

The second implication is the potentially misleading ranking of metals in terms of their ecotoxicological hazard, when CTPs are derived using just effect data retrieved from experiments carried out in OECD soils. CTP must ensure a fair comparison between substances in terms of their potential impact on an ecosystem. This ranking will naturally depend on soil type and properties (hence spatially-explicit CTPs are needed), but for global-scale impact assessment average or median CTP values calculated for a wide range of natural soils are used in cases when the emission source is not known (Owsianiak et al. 2013; Dong et al. 2014). CTP values calculated using just OECD-based soils will, however, rank metals for which ecotoxicity is lowered by Ca^{2+} (which is ca. 1 order of magnitude larger in OECD soil pore water compared to natural soils), as being less toxic than they are in natural soils. These metals include Cu, Ni, Cd and Co (Ardestani et al. 2014 and references therein). Only for metals not influenced by dissolved Ca^{2+}, a hypothetical OECD-based HC50 could be a sufficient indicator for use in global-scale assessments, like traditional site-generic LCA. As ionic composition of pore water is important for many metals, however, we recommend experimentalists measuring and reporting concentration of base cations and considering them in soil ecotoxicity experiments.

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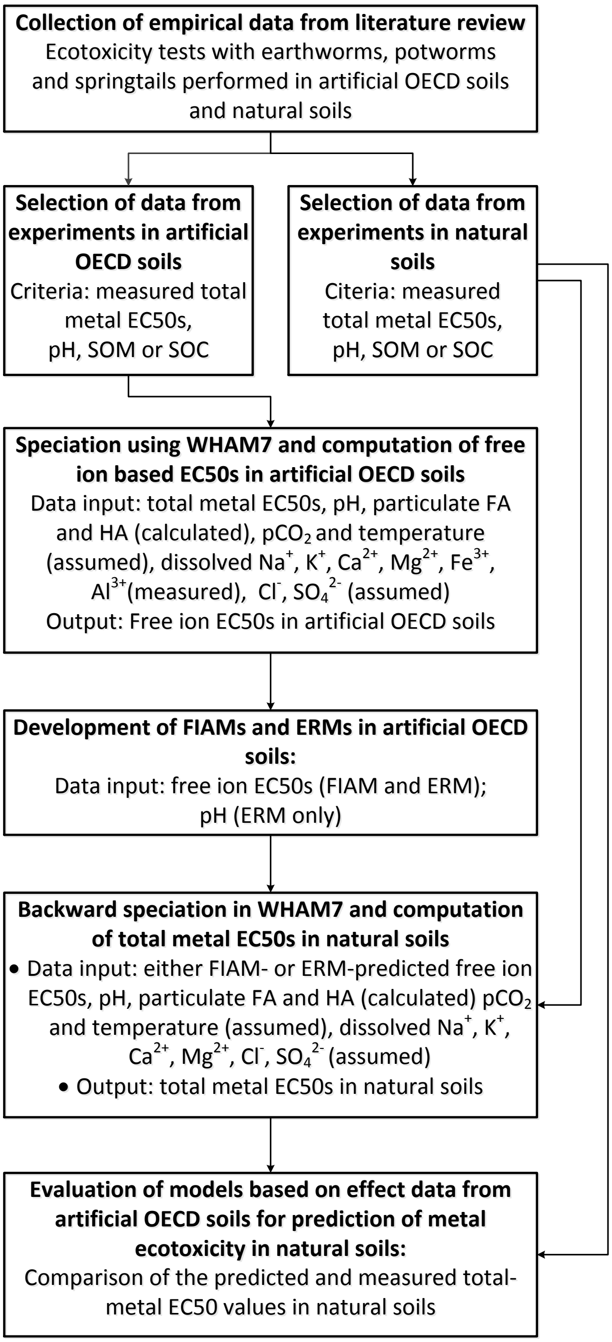

Fig. 1. Study design. EC50 - concentration with (lethal) effects in 50% of the individuals of a species, FIAM – Free Ion Activity Model, ERM – Empirical Regression Model, FA – fulvic acids, HA – humic acids, pCO_2 – atmospheric partial pressure of CO_2, SOM – soil organic matter, SOC – soil organic carbon, WHAM7 - Windermere Humic Aqueous Model 7.

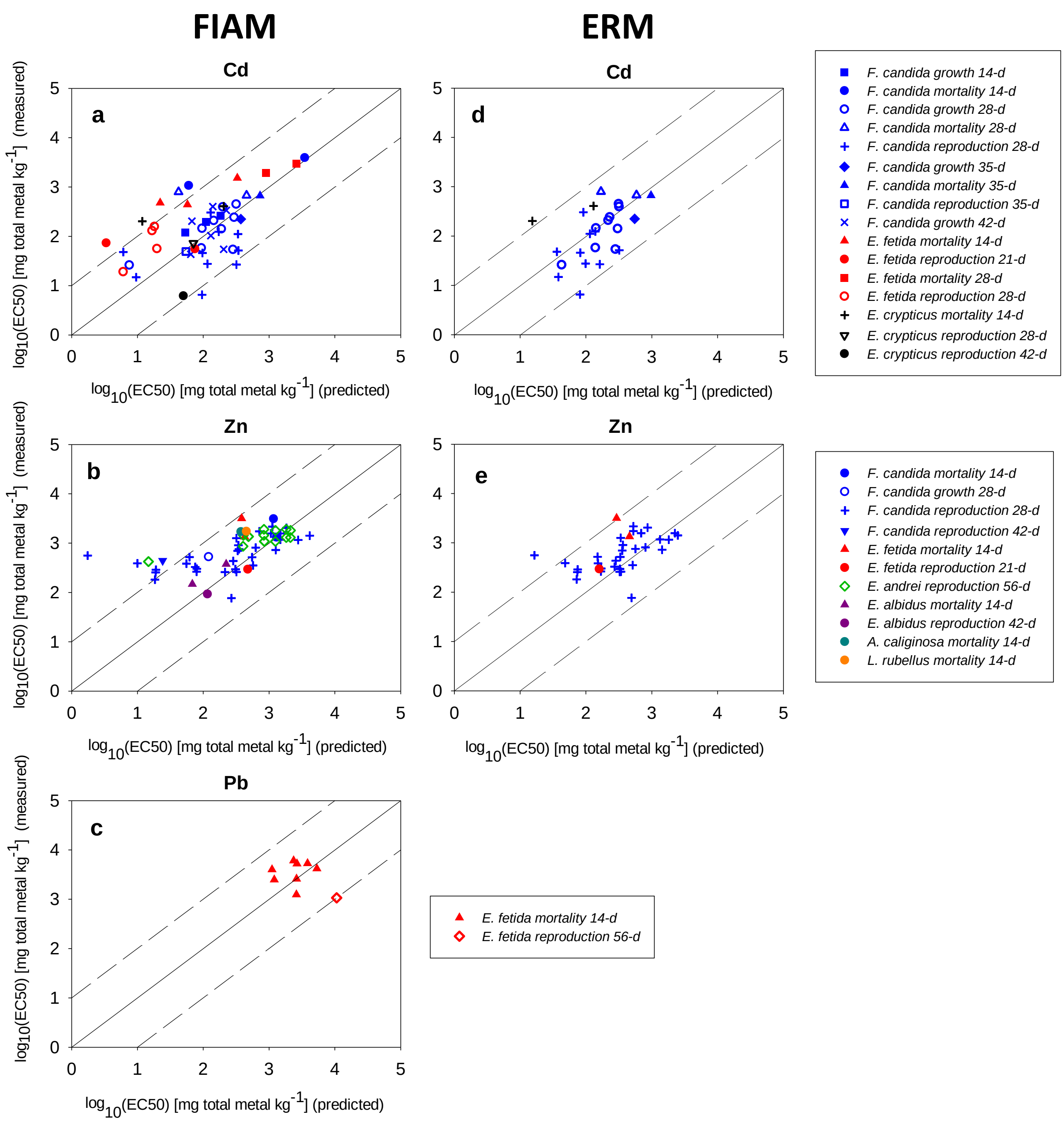

Fig. 2. Performance of FIAM (Free Ion Activity Model) developed using effect data measured in artificial OECD soils for prediction of metal ecotoxicity in natural soils (a-c) and performance of three types of ERM (Empirical Regression Model) developed using effect data measured in artificial OECD soils for prediction of metal ecotoxicity in natural soils (d, e). The dashed lines represent deviations equal to 1 order of magnitude. Statistical details of FIAMs' and ERMs' performance are presented in Appendix A5, Tables A7-A9.

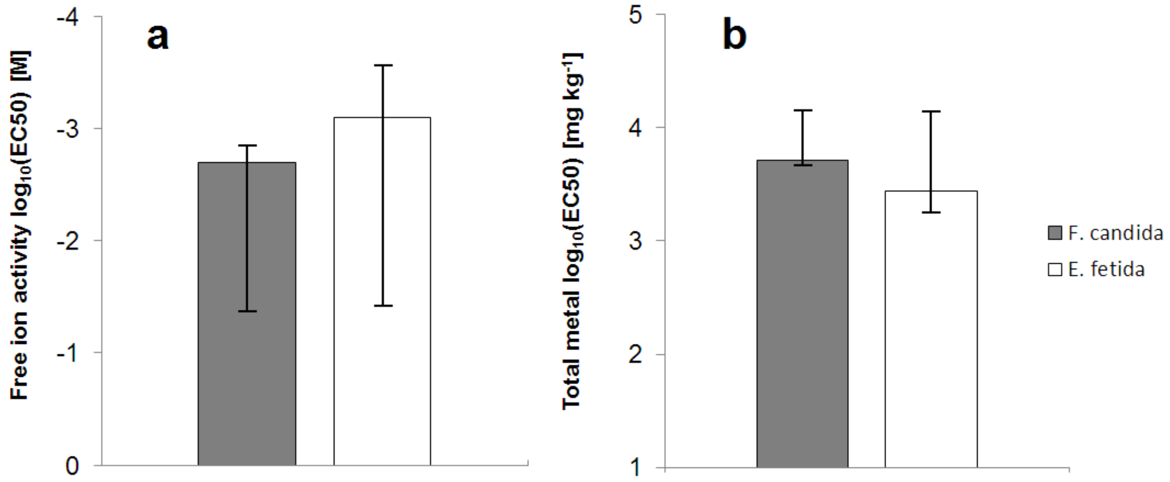

Fig. 3. Variability in EC50 of Cd to to *E. fetida* (2-d mortality) and *F. candida* (7-d mortality) as influenced by concentrations of dissolved base cations of Cd, basing on (a) free ion based EC50 computed using TBLMs and (b) total metal based EC50 as predicted using the TBLM-based free ion EC50. The bars represent $\log_{10}$(EC50) values calculated for median values of dissolved base cations. The error bars represent $\log_{10}$(EC50) calculated for 2.5th, or 95th percentile values of dissolved base cations for 760 soils from around the World (Owsianiak et al. 2013). Terrestrial biotic ligand binding constants are (i) *E. fetida* ($LogK_{Me\text{-}BL}$ = 4.00; $LogK_{Ca\text{-}BL}$ = 3.35; $LogK_{Mg\text{-}BL}$ = 2.82; $LogK_{Na\text{-}BL}$ = 1.57; $LogK_{K\text{-}BL}$ = 2.31; $LogK_{H\text{-}BL}$ = 5.41; $f_{BL\text{-}50}$ = 0.72) (Li et al. 2008) and (ii) *F. candida* ($LogK_{Me\text{-}BL}$ = 1.62; $LogK_{Ca\text{-}BL}$ = 2.87; $LogK_{H\text{-}BL}$ = 4.97; $f_{BL\text{-}50}$ = 0.038) (Ardestani et al. 2013). EC50 - the concentration with effects in 50% of the individuals of a species.

Table 1. The summary of collected ecotoxicity data showing the range of collected total metal EC50s (the concentrations with effects in 50% of the individuals of a species), geometric means of pH, and arithmetic means for SOC (soil organic carbon). Details of the collected data are presented in the Appendix A2, Tables A1-A6.

Metal	Number of data points	Number of species	Number of studies	The range of total metal EC50s across all tested organisms (min – max) [mg kg^{-1} soil]	mean pH_{H2O} (min – max)	mean SOC (min – max) [%]
Artificial OECD soils						
Cd	233	13[a]	38	2.83 – 4730	6.46 (4.14 – 8.18)	4.77 (0.00 – 7.87)
Pb	54	6[b]	15	40.30 – 12000	5.99 (4.00 – 7.00)	4.16 (0.00 – 5.62)
Zn	138	11[c]	28	3.78 – 5150	6.31 (4.00 – 7.90)	4.78 (0.00 – 8.43)
Natural soils						
Cd	70	3[d]	12	6.20 – 3930	5.87 (3.80 – 7.76)	3.04 (0.63 – 12.19)
Pb	30	2[e]	10	181.00 – 6050	6.71 (4.50 – 8.44)	2.64 (0.70 – 11.24)
Zn	98	6[f]	17	35.00 – 7264	5.96 (3.86 – 7.90)	3.65 (0.84 – 51.69)

[a] *Eisenia fetida, Enchytraeus albidus, Enchytraeus crypticus, Enchytraeus doerjesi, Folsomia candida, Fridericia peregrinabunda, Lobella sokamensis, Lumbricus rubellus, Onychiurus yodai, Paronychiurus kimi, Sinella umesaoi, Sinella coeca, Sinella curviseta.*

[b] *Eisenia fetida, Enchytraeus albidus, Folsomia candida, Paronychiurus kimi, Pheretima guillelmi, Sinella coeca.*

[c] *Aporrectodea caliginosa, Eisenia andrei, Eisenia fetida, Enchytraeus albidus, Enchytraeus crypticus, Enchytraeus doerjesi, Folsomia candida, Lobella sokamensis, Lumbricus rubellus, Orthonychiurus pseudostachianus, Pheretima guillelmi.*

[d] *Eisenia fetida, Enchytraeus crypticus, Folsomia candida.*

[e] *Eisenia fetida, Folsomia candida.*

[f] *Aporrectodea caliginosa, Eisenia andrei, Eisenia fetida, Enchytraeus albidus, Folsomia candida, Lumbricus rubellus*

Table 2. Free ion activity models (FIAMs) and empirical regression models (ERMs) developed basing on effect data measured in artificial OECD soils. GCV is geometric coefficient of variation, R^2 is coefficient of determination, se is standard error of estimation, p is the probability level. ERMs were developed using at least 5 independent data points, thus, the number of points is smaller compared to FIAMs and no ERM for Pb could be developed. EC50 is the concentration with effects in 50% of the individuals of a species.

Species and ecotoxicity endpoint	Geometric mean of free ion activity EC50 [$mol/L_{pore\ water}$]	GCV [%]	Geometric mean of total metal EC50 [mg kg_{soil}^{-1}]	GCV [%]	*n*	Empirical regression $\log_{10}[EC50] = a \times pH + b$ EC50 values expressed as [$mol/L_{pore\ water}$]	R^2	se	*p*	*n*
Cd										
E. fetida mortality 14-d	8.4E-06	208	974	139	9	n.d.	-	-	-	-
E. fetida reproduction 21-d	1.5E-06	106	179	104	2	n.d.	-	-	-	-
E. fetida mortality 28-d	3.1E-06	-	588	-	1	n.d.	-	-	-	-
E. fetida reproduction 28-d	9.0E-08	93	20	129	3	n.d.	-	-	-	-
E. crypticus mortality 14-d	4.9E-06	418	100	549	38	$\log_{10}[EC50] = -0.21 \times pH - 4.25$	0.12	0.49	0.21	15
E. crypticus reproduction 28-d	1.4E-06	-	158	-	1	n.d.	-	-	-	-
E. crypticus reproduction 42-d	1.1E-06	-	130	-	1	n.d.	-	-	-	-
F. candida growth 14-d	1.1E-06	14	270	11	3	n.d.	-	-	-	-
F. candida mortality 14-d	2.1E-05	968	1460	66	4	n.d.	-	-	-	-
F. candida growth 28-d	2.1E-06	230	309	65	7	$\log_{10}[EC50] = -0.40 \times pH - 2.97$	0.81	0.27	0.04	5
F. candida mortality 28-d	8.9E-06	169	1014	51	8	$\log_{10}[EC50] = -0.36 \times pH - 2.70$	0.88	0.19	0.02	5
F. candida reproduction 28-d	2.7E-06	607	201	167	12	$\log_{10}[EC50] = -0.54 \times pH - 2.34$	0.72	0.46	0.03	6
F. candida growth 35-d	6.2E-06	148	525	44	17	$\log_{10}[EC50] = -0.38 \times pH - 2.75$	0.93	0.12	<0.01	14
F. candida mortality 35-d	1.4E-05	170	842	44	12	$\log_{10}[EC50] = -0.45 \times pH - 2.07$	0.84	0.19	<0.01	10
F. candida reproduction 35-d	6.3E-07	64	129	35	6	n.d.	-	-	-	-
F. candida growth 42-d	1.4E-06	101	328	82	12	n.d.	-	-	-	-
Pb										
E. fetida mortality 14-d	2.2E-07	342	3216	181	4	n.d.	-	-	-	-
E. fetida reproduction 56-d	6.9E-08	-	1940	-	1	n.d.	-	-	-	-
Zn										
A. caliginosa mortality 14-d	4.4E-06	-	561	-	1	n.d.	-	-	-	-
E. andrei reproduction 56-d	1.3E-05	0	1731	0	2	n.d.	-	-	-	-

E. fetida mortality 14-d	4.7E-06	75	857	43	7	$\log_{10}$[EC50] = −0.28 × pH – 3.49	0.21	0.21	0.37	5
E. fetida reproduction 21-d	6.7E-06	243	336	74	16	$\log_{10}$[EC50] = −0.50 × pH – 2.41	0.24	0.24	0.02	5
E. albidus mortality 14-d	1.7E-06	-	566	-	1	n.d.	-	-	-	-
E. albidus reproduction 42-d	7.6E-07	54	247	23	8	n.d.	-	-	-	-
F. candida mortality 14-d	5.5E-05	-	5150	-	1	n.d.	-	-	-	-
F. candida growth 28-d	4.0E-06	11	1217	1	3	n.d.	-	-	-	-
F. candida reproduction 28-d	8.5E-06	574	429	135	20	$\log_{10}$[EC50] = −0.42 × pH – 2.48	0.35	0.35	<0.01	10
F. candida reproduction 42-d	3.1E-06	74	635	11	2	n.d.	-	-	-	-
L. rubellus mortality 14-d	5.9E-06	-	728	-	1	n.d.	-	-	-	-