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SUMMARY REPORT

TERRESTRIAL METALS BIOAVAILABILITY: A LITERATURE-DERIVED CLASSIFICATION PROCEDURE FOR ECOLOGICAL RISK ASSESSMENT

Ecological Risk Assessment Support Center
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
AICc	Akaike Information Criterion with small-sample bias correction
BAF	bioaccumulation factor
BCF	bioconcentration factor
C	carbon
$CaCl_2$	calcium chloride
CART	classification and regression trees
Cd	cadmium
CEC	cation exchange capacity
CLAY	variable that refers to soil particle sizes < 2 μm
Co	cobalt
DOSE	nuisance variable pertaining to the concentration the metal was either dosed or measured in field-contaminated soils
EC_X	effective concentration for X% of the population
Eco-SSL	Ecological Soil Screening Level
ENDPOINT	nuisance variable that specifies the measurement attribute of the organism
EPC	Exposure Point Concentration
ERA	ecological risk assessment
ERAF	Ecological Risk Assessment Forum
KCl	potassium chloride
LC_X	lethal concentration for X% of the population
LOEL	lowest-observed-effect level
LOO	leave-one-out
MAXDEPTH	variable that refers to the complexity of the regression tree. See text
METAL	nuisance variable that specifies the particular metal that was evaluated
Ni	nickel
nl	natural log
NOEL	no-observed-effect level
PARAMETER	nuisance variable that specifies the reported toxicity benchmark
Pb	lead
RECEPTOR	nuisance variable that specifies among plant, invertebrates, or microbes

LIST OF ABBREVIATIONS (continued)

RT	regression tree
SE	standard error
SOM	soil organic matter
SPECIES	nuisance variable that specifies the particular species that was evaluated
SSD	species sensitivity distribution
STUDY	nuisance variable that specifies study to be treated as a random effect
TYPE	nuisance spiked versus field-contaminated soils
Zn	zinc

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PREFACE

A request was submitted by the Ecological Risk Assessment Forum (ERAF) to the Office of Research and Development's Ecological Risk Assessment Support Center (ERASC) relating to the issue of terrestrial metals bioavailability. The ERAF specifically requested a product that characterizes typical aerobic soils in terms of their potential to mitigate metals bioavailability to soil-dwelling biota in response to increased pressure from the academic community to explicitly incorporate bioavailability concepts into the ecological risk assessment (ERA) process. An exhaustive literature search and corresponding meta-analysis of the empirical data was recommended and performed. The result is a quantitative tool that broadly accounts for metals bioavailability and is proposed to augment the ERA process and risk-based remediation of metals-contaminated soils. The tool, or classification procedure, presented here is suggested to be used with other analyses such as direct toxicity testing of contaminated soils. The present document summarizes the derivation and potential use of this tool as described in the ERASC draft response and a peer-reviewed article (Anderson et al., 2013).

EXECUTIVE SUMMARY

Interstudy variation among bioavailability studies is a primary deterrent to a universal methodology to assess metals bioavailability to soil-dwelling organisms and is largely the result of specific experimental conditions unique to independent studies. The primary objective of this review is to synthesize information in the open literature on the effects of soil chemical/physical properties on metals bioavailability independent of extraneous variation due to the specific attributes of individual studies. Accordingly, two data sets were established from relevant literature; one includes data from studies related to bioaccumulation (total obs = 520), while the other contains data from studies related to toxicity (total obs = 1,264). Experimental factors that affect bioavailability independent of the effect of soil chemical/physical properties were considered nuisance variables, i.e. variables not of direct interest in the context of this study, but that need to be considered in analyzing the data. Variation associated with significant nuisance variables was statistically apportioned from the variation attributed to soil chemical/physical properties for both data sets using a linear mixed model. Residual bioaccumulation data were

then used to develop a nonparametric regression tree whereby bootstrap and cross-validation techniques were used to internally validate the resulting classification procedure. A similar approach was employed with the toxicity data set as an independent external validation. These analyses obviously emphasize bioaccumulation as the primary metric for assessing bioavailability but demonstrate concurrence with studies on toxicity. The validated classification procedure is proposed as a quantitative tool that broadly characterizes typical aerobic soils in terms of their potential to sequester common divalent cationic metal contaminants and mitigate their bioavailability to soil-dwelling biota. This classification procedure is proposed to augment other ERA approaches.

INTRODUCTION

Bioavailability of metal contaminants has been given much attention over the last decade. In particular, a plethora of research has demonstrated that the uptake and subsequent effects of toxic trace elements to soil-dwelling organisms are largely regulated by the specific chemical/physical composition of contaminated soils. Despite these recent advances in assessing terrestrial metals bioavailability (Scheckel et al., 2009), a disparity exists between the state of the science and regulatory practice in terms of *explicitly* incorporating methods to assess bioavailability into the ecological risk assessment (ERA) framework for metals. Although the European Union has developed risk-based ecological soil standards using bioavailability models derived from empirical relationships (Smolders et al., 2009; Semennzin et al., 2007), the United States has yet to adopt a parallel approach (see U.S. EPA, 2007). In general, a universally applicable methodology that accounts for metals bioavailability would augment the ERA process and risk-based remediation of metals-contaminated soils.

Several methods are commonly applied to assess metals bioavailability. Sequential-, parallel-, and single-chemical extractions characterize metal concentrations among various soil geochemical phases. Relative advantages and disadvantages of chemical extraction methods are reviewed elsewhere (Rao et al., 2008; Gleyzes et al., 2002). In general, however, current extraction methods are considered insufficient to accurately assess metals bioavailability simultaneously to multiple ecological receptors among heterogeneous soils (Peijnenburg et al., 2007). Moreover, chemical extractants can modify chemical speciation and soil solution chemistry, resulting in operationally defined phases with measured metals concentrations that may not correlate with biological responses (Scheckel et al., 2009; 2003).

Alternatively, mechanistic models are predicated on theoretical thermodynamic and kinetic principles, and most predict biological effects of metals exposure. Primary mechanistic models applicable to terrestrial metals bioavailability include the free ion activity model (Hough et al., 2005; Lofts et al., 2004; Parker and Pedler, 1997), the terrestrial biotic ligand model (Thakali et al., 2006a; Thakali et al., 2006b; Steenbergen et al., 2005), and soil-water metal equilibrium partitioning (Degryse et al., 2009). Mechanistic models are essential to a comprehensive understanding of biological responses to soil contamination and have been correlated with measurements from field-contaminated soils (Koster et al., 2006; Parker et al., 2001). However, application of mechanistic models can involve complicated input parameters (often modeled from theoretical functions that assume equilibrium conditions), which could limit their utility in regulatory practice.

Bioavailability models predicated on empirical data, on the other hand, normalize experimental bioaccumulation or toxicity estimates from bioassays among contrasting soils (e.g., Criel et al., 2008; Rooney et al., 2007; Oorts et al., 2006). Bioassays with soil-dwelling organisms are implicit indicators of bioavailability because they reflect cumulative mechanistic soil biogeochemical processes (Basta et al., 2005; Lanno et al., 2004). Empirical models quantify these processes, as well as random variability, unlike mechanistic models. Individual empirical models presented throughout the open literature, although applicable to the specific experimental conditions, may not provide tenable prediction across the entire range in contaminated soils types. Moreover, preferentially utilizing a particular model in lieu of others available, all else equal, may be programmatically difficult to justify.

Despite the preponderance of studies demonstrating systematic differences in uptake and toxicity among contaminated soils, universal regulatory acceptance of a methodology to assess metals bioavailability to soil-dwelling organisms is impeded in large part by irreproducibility among studies, leading to the overarching issue of uncertainty (SERDP and ESTCP, 2008). The extent to which inconsistencies in the literature can be resolved by meta-analysis of the empirical data merits further investigation, given numerous experimental artifacts among independent studies (e.g., Lowe and Butt, 2007; Sochova et al., 2006; Clark et al., 2004; Crouau and Cazes, 2003) and variations in standard test methods. Many standard test methods exist for terrestrial toxicity testing (e.g., Environment Canada, 2005; ISO, 2005; ASTM, 2004; U.S. EPA, 1994). Selection of test species, range of contaminant doses, measurement endpoint, and numerous other experimental conditions are highly variable among studies and produce artifacts at the discretion of the researcher. Moreover, comparison of exposure studies, as related to bioavailability, is operationally challenged by the same issues. Application of current bioavailability models, regardless of the metric used to assess bioavailability, is limited to a specific suite of biotic and abiotic experimental conditions.

One method employed to evaluate results from multiple studies on metals bioavailability is a modification of the species sensitivity distribution (SSD) approach (Smolders et al., 2009; Semennzin et al., 2007). In a typical SSD, usually the 5^{th} or 1^{st} percentile of toxicity parameters, which reflect media-specific contaminant concentrations, from the cumulative probability distribution of a sample of species is obtained (usually from the literature) and is considered protective of 95 or 99% of species, respectively (Posthuma et al., 2002). In the context of bioavailability, toxicity parameters used in the SSD can be normalized based on regression models that include soil chemical/physical properties as predictors (Smolders et al., 2009; Semennzin et al., 2007). Additional study-specific variation uncontrolled among bioavailability studies (e.g., metal, endpoint, dose, etc.), however, should be accounted for, as well as interspecies variation. Moreover, the effects-based SSD approach ignores results of studies that report bioaccumulation as the metric of bioavailability. A meta-analysis that utilizes data with both bioaccumulation and toxicity metrics that effectively apportions "nuisance variation" from the effect of soil chemical/physical properties is paramount to accurately synthesize the literature on terrestrial metals bioavailability universally applicable to ERAs.

The primary objective of this review is to quantify soil chemical/physical property effects on terrestrial metals bioavailability by meta-analysis, controlling the confounding influence of the specific attributes of individual studies independent of soil metal sequestration. In this context, bioavailability is defined, simplistically, as the fraction of a total metal load (in soil) that has the potential to traverse a biological membrane. In fact, membrane transport is inherent in most, if not all, interpretations of bioavailability (Drexler et al., 2003). It follows that body burden (i.e., bioaccumulation) is the appropriate metric for assessing bioavailability because trace metals usually achieve steady-state concentrations in most soil-dwelling organisms, especially essential trace elements (Mleczek et al., 2009; Nahmani et al., 2009; Vijver et al., 2001; Peijnenburg et al., 2000; Spurgeon and Hopkin, 1999). However, the majority of literature on the subject utilizes endpoints that reflect toxicity. Consequently, two data sets were established from relevant literature; one includes information from studies related to bioaccumulation (total obs = 520), while the other contains studies related to toxicity (total obs = 1,264). Experimental factors that affect bioavailability independent of the effect of soil chemical/physical properties were considered nuisance variables. Variation associated with significant nuisance variables was statistically apportioned from the variation attributed to soil chemical/physical properties for both data sets using a linear mixed model. Residual bioaccumulation data were then used to develop a nonparametric regression tree whereby bootstrap and cross-validation techniques were used to internally validate the resulting classification procedure. A similar approach was employed with the toxicity data set as an independent external validation. These analyses obviously emphasize bioaccumulation as the primary metric for assessing bioavailability but simultaneously tests concurrence with studies on toxicity.

METHODS

DATA SETS

Two independent data sets were compiled from published studies on the effects of abiotic soil factors on the bioavailability of the common divalent cationic metals cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) to soil-dwelling organisms available in the open literature. Only studies that included multiple soil types were considered in order to limit the scope of the review exclusively to studies that were designed to investigate metals bioavailability in some capacity. The only exception was that all the data used in the development of plant and invertebrate Ecological Soil Screening Levels (Eco-SSLs) for Cd, Co, Cu, Ni, Pb, and Zn (U.S. EPA, 2005) were included since those metals were already being considered. Applicable studies for additional metals were lacking. Some studies (e.g., Zhao et al., 2006) were excluded because results were presented in a form such that did not allow extraction. Most studies that reported internal tissue concentrations also reported bioaccumulation factor (BAF) values computed as ratios of tissue metal concentration to total soil concentration determined by vigorous acid digestion. In this context, BAF is used interchangeably with bioconcentration factor (BCF) values. If BAF values were not reported, they were computed and used for all analyses, which is comparable to analyses based on tissue concentrations, given the objectives. For studies employing a dose-response design, reported BAFs reflect a linear regression slope.

The data sets comprehensively summarize bioaccumulation (i.e., BAF values) and acute toxicity (i.e., no-observed-effect level [NOEL], lowest-observed-effect level [LOEL], effective concentration for 10% of the population [EC_{10}], EC_{20}, EC_{50}, and lethal concentration for 50% of the population [LC_{50}] values) across 122 and 131 contaminated natural soils, respectively. However, due to co-use of select soils among multiple studies, a total of 189 independent soils were collated across both data sets. The bioaccumulation data set contains BAF values for

11 plants, 2 earthworms, and 1 springtail, while the toxicity data set contains toxicity values for 19 plants, 20 earthworms, 1 springtail, and microbes. In addition to soil characterization variables, both data sets were developed with generalized variables ubiquitous to all studies; METAL, SPECIES, ENDPOINT, RECEPTOR (i.e., plant, invertebrate, or microbe), and TYPE (i.e., spiked vs. field-contaminated soils). The bioaccumulation data set also contains a variable (DOSE) pertaining to the concentration the metal was either dosed or measured in field-contaminated soils—by evaluating DOSE on BAF values as opposed to tissue concentrations, nonlinear effects could be assessed more easily. If the study had a dose-response design, the value of DOSE that was recorded is the highest dosed concentration. The toxicity data set exclusively contains dose-response studies and a variable (PARAMETER) that reflects the reported toxicity benchmark (e.g., NOEL, LOEL, EC_{10}, etc.). All other data were considered too inconsistent or sparse to consider applicable for coding and analyses.

Soil characterization data were highly inconsistent among studies, yielding only as many as three matrix properties common to most studies; pH, clay content, and either organic carbon (C) or soil organic matter (SOM). Consequently, these were the properties selected to evaluate and were assumed to generally account for the cumulative and inherently interactive processes of metal sequestration and attenuation (Hamon et al., 2007); although it is well known that metals have variable affinities for soil geochemical phases (e.g., Saeki and Kunito, 2009), certain generalizations regarding metals bioavailability can be made. In general, precipitation and solid phase adsorption are primary mechanisms mitigating metals bioavailability, but the magnitude of sequestration largely depends on both direct and indirect effects of soil reactivity; formation constants vary as a function of soil pH among metal-bearing minerals, and ionized organic and inorganic complexation sites within the soil matrix increase with soil pH due to deprotonation (Sparks, 2003). Whereas, soil pH influences bioavailability directly by regulating the formation of additional solid phase sorbents, such as carbonate minerals (Sipos et al., 2009, 2008), and the chemical form of the metal species (Lofts et al., 2005; Nolan et al., 2003).

Admittedly, omission of other soil matrix properties that have been shown to interact with metal solubility, thereby influencing bioavailability (e.g., amorphous oxides [e.g., Dayton et al., 2006; Bradl, 2004; Peijnenburg et al., 1999a] and cation exchange capacity [CEC] [e.g., Anderson and Basta, 2009b; Criel et al., 2008; Rooney et al., 2006]) is an oversimplification of soil metal sequestration. However, soil CEC, when measured at ambient soil pH, is usually correlated with total clay content and SOM because ionized organic functional groups and aluminosilicate clay edges (both of which contribute to overall soil CEC) are pH dependent (Ge and Hendershot, 2002; Basta et al., 1993). Also, amorphous oxide data (see McKeague and Day, 1993) were only reported in a small subset of studies. To preserve symmetry among the data sets, promoting an unbiased quantitative assessment of the available data, only studies that reported all three selected soil properties were utilized. The ranges in selected soil properties within the published studies have been compiled for both data sets and are summarized in Table 1.

DATA MANAGEMENT AND ASSUMPTIONS

Certain assumptions and conversions were necessary to accommodate variations in the design and reporting among studies. Primary assumptions herein include (1) reported measurements among all studies reflect steady-state toxicokinetics and (2) exposure occurs through direct absorption (or adsorption if bioreactive upon contact) or dietary uptake (e.g., soil invertebrates). All earthworms were depurated, but depuration and exposure times were also assumed not to affect BAF values. Concentrations, including toxicity values, are on a dry weight-basis and, if necessary, were converted to mg kg^{-1}. Additionally, if organic C values were reported, they were doubled to estimate SOM values according to an approximation of the organic C content of SOM (Sleutel et al., 2007). Then, if necessary, SOM values were converted to percentages as were clay content values. All pH values reflect either potassium chloride (KCl) or calcium chloride ($CaCl_2$) extracts and were analyzed indiscriminately. No pH measurements in deionized water were recorded.

APPORTIONMENT OF NUISANCE VARIATION

The term *nuisance variable* refers to uncontrolled experimental conditions that influence BAF and/or toxicity measurements independent of the effect of soil metal sequestration. For example, within a soil type, bioaccumulation is expected to be influenced by toxicity at higher doses depending on the metal, species, and other factors. The term *apportionment* refers to the statistical methodology whereby variation attributed to significant nuisance variables is quantitatively partitioned from the effect associated with soil chemical/physical properties.

Effects of nuisance variables were evaluated statistically using a linear mixed model. This approach allows a choice in how to represent the effects of a given variable, as either a random source of variation (e.g., among species) or as a set of fixed effects (e.g., of individual species. Selection of nuisance variables to be included in the model was based on the Akaike Information Criterion (AIC) with small-sample bias correction (AICc, Burnham and Anderson, 2002). The criterion can be applied in two ways (see Table 2). The single model with best (lowest) AICc has been here termed the "most parsimonious model," because of penalization of fit for the number of variables included in the model. Alternatively, the use of AICc model weights may allow some consideration of model uncertainty in the selection of a single best model. Additional details of the methodology are contained in Anderson et al. (2013).

CLASSIFICATION PROCEDURE DEVELOPMENT AND VALIDATION

Residual values (observed values of log BAF minus predicted values) from the best supported linear model describing nuisance variation were recovered for both data sets. Variation in residual values is assumed to be attributed solely to bioavailability differences among soils and random or latent error. Residuals from the bioaccumulation data set were used as the response variable in the development of a regression tree (RT) methodology. The RT methodology is essentially a case of the general classification and regression tree (CART) algorithm of Breiman et al. (1984), also see Ripley (1996), as implemented with the rpart() package Version 3.1-42 (Therneau and Atkinson, 2010; Faraway, 2006), with specially-programmed extensions (R Development Core Team, 2008). An extension of the methodology was to compute bootstrap support for particular variables, equal to percentages of bootstrap samples where a variable was selected in the CART algorithm. Bootstrap samples were of the set of studies (all data or no data from a given study were selected into a given bootstrap sample). While the CART algorithm is relatively well established, a brief summary of the standard algorithm, and our specific implementation, is provided in Anderson et al. (2013).

RESULTS AND DISCUSSION

APPORTIONMENT OF NUISANCE VARIATION

Mixed-model analysis of the nuisance variables resulted in four candidate models for the bioaccumulation data set and three candidate models for the toxicity data set with cumulative Akaike weights >99% (see Table 2). All models presented in Table 2 are considered significant, given that the null models (i.e., intercept only) were also evaluated (Burnham and Anderson, 2002). The additive fixed effects of the variables METAL, RECEPTOR, ENDPOINT, and PARAMETER resulted in the most parsimonious model for the toxicity data set and accounted for 91% of the overall model weight. The most parsimonious model for the bioaccumulation data set coincidentally also accounted for 91% of the overall model weight and contained the variables METAL, ENDPOINT, DOSE, and the METAL × DOSE interaction. All four of the candidate bioaccumulation models contained the two-way METAL × DOSE interaction, reflecting a relatively strong influence on BAF values (see Table 2). Significant random variation due to SPECIES was observed for both bioaccumulation ($p = 0.0189$) and toxicity ($p = 0.0247$) data sets, whereas, significant interstudy heterogeneity was only observed in the toxicity data set ($p = 0.0129$) as determined by variance component estimation (Lindsey, 1997).

The variable TYPE only occurred in the third best model for the bioaccumulation data set, which only accounted for 3% of the overall model weight (see Table 2). Although there is evidence to conclude that the contamination source may have influenced BAF values, the effect was minor relative to other experimental variables evaluated, similar to the results of Peijnenburg et al. (2000). Most researchers subject artificially contaminated soils to various wet-dry cycles to simulate aging, attenuating the effect of the metal (Orrono and Lavado, 2009; Si et al., 2006). Metal salt-amended soils that are sufficiently “aged” can produce similar ecotoxicological effects of field-contaminated soils (Smolders et al., 2009). Subsequent discussion and analyses pertain to the most parsimonious models (see Table 2).

Bioaccumulation

Variation in BAF values was predominantly apportioned by metal-stratified doses or the METAL × DOSE model parameter. In general, mean predicted intrametal BAF values decreased with increasing dose, indicating constant (or declining) internal concentrations with increasing total soil concentrations (see Figure 1). Metal accumulation is related to metal solubility/speciation (McLaughlin, 2002), membrane transport (Welch and Norvel, 1999; Kochian, 1993), and uptake kinetics (Nahmani et al., 2009; Peijnenburg et al., 2000) and declines with saturation of detoxification mechanisms, resulting in inherent toxicity thresholds that can be quantified (Anderson et al., 2008). Threshold metal accumulation is referred to as the critical body residue (Ma, 2005; McCarty and Mackay, 1993) and correlates with the onset of a toxic response (Conder et al., 2002; Lanno et al., 1998). Critical body residues can impede further metal accumulation at toxic doses (e.g., Hasaan et al., 2009; Kumar et al., 2008). Obviously, toxicity affects BAF values and is usually the reason for the "plateau effect" in metal salt-amended soils (McLaughlin, 2002; Hamon et al., 1999). Although linear metal accumulation has been reported in plants and soil invertebrates at steady state (e.g., Yanai et al., 2006; Spurgeon and Hopkin, 1996), results usually reflect subtoxic soil concentrations. Toxicity-induced nonlinear metal accumulation has also been demonstrated in many exposure studies (e.g., Anderson and Basta, 2009b; Smilde et al., 1992).

Differences among SPECIES and ENDPOINT further apportioned variation in BAF values. Approximately half of the bioaccumulation data set contains BAF values for vascular plants. Among these, only two studies reported endpoints other than metal accumulation in aboveground biomass; one evaluated metal accumulation in the grain of several agronomic species (Smilde et al., 1992), while the other evaluated cumulative metal levels in the shoots and roots of *Avena* sp. (Bjerre and Schierup, 1985). Conversely, BAF values for soil invertebrates reflect whole body residues. So, SPECIES essentially contrasts aboveground bioaccumulation among plants and whole body residues among soil invertebrates, which confounded evaluation of RECEPTOR and ENDPOINT differences. Mean predicted BAF values were in the following order: aboveground plant biomass < soil invertebrate whole body residues (see Figure 2). Thus, although plants and soil invertebrates may be surrogate receptors for estimating potential terrestrial metals exposure (Scott-Fordsmand et al., 2004), results depend on the vegetative tissue analyzed and/or the specific metal-sensitivity of the species evaluated.

Toxicity

As expected, variation in toxicity values was apportioned by METAL. Higher predicted toxicity values reflect relatively less toxicity because higher total soil concentrations were required for equivalent toxicity. Thus, the rank order of mean predicted toxicity values illustrates the observed relative potencies of the six metals evaluated in order of least potent to most potent. Mean predicted toxicity values were in the order Cu = Pb = Zn > Ni > Co > Cd (see Figure 3). Cadmium toxicity is expressed in numerous endpoints in plants (Hasaan et al., 2009) and soil invertebrates (Roh et al., 2006) and is routinely reported as a relatively potent cationic trace metal in ecological toxicity testing (e.g., Anderson and Basta, 2009b; Athar and Ahmad, 2001; Bowers et al., 1997). Predicted metal potencies are roughly consistent with trends in Eco-SSLs for plants and soil invertebrates (U.S. EPA, 2005).

Moderate variation in toxicity values was apportioned by ENDPOINT. Although some endpoints are specific to a receptor, evaluating them independently, in conjunction with RECEPTOR, accounted for the most variation. As expected, reproduction-related endpoints (cocoon and grain production) were the most sensitive, while mortality (LC_{50} values) was the least sensitive (see Figure 4). Studies with microbial endpoints (e.g., nitrification, mineralization, and respiration) tended to be relatively sensitive, underscoring their relevance to bioavailability studies, which have become commonplace in the literature (e.g., Magrisso et al., 2009; Oorts et al., 2006; Smolders et al., 2004; Giller et al., 1999).

Obviously, the reported toxicity parameter from a dose-response curve reflects the magnitude of an effect. Consequently, PARAMETER was crucial to apportioning nuisance variation (see Figure 5). As expected, LC_{50} values were the least sensitive, coinciding with the mortality endpoint. However, an unexpected result was that, on average, mean predicted EC_{10} values were lower than mean predicted NOEL and LOEL values. EC_{10} values are estimated from fitted dose-response curves while NOEL and LOEL values are ordinarily determined by statistical comparisons of treated to negative control groups (Eaton and Klaassen, 2001), with possibly limited sensitivity. Thus, EC_{10} estimates may be lower than NOEL values suggesting that quantitative dose-response evaluation can be a more conservative methodology, especially when low-dose values are scant.

The variable RECEPTOR was a relatively minor source of nuisance variation. Mean predicted toxicity values among receptors were in the following order:
microbes < plants < springtails < earthworms (see Figure 6). Thus, in general, microbes tend to produce more conservative toxicity estimates when endpoint, metal, and toxicity parameters have been accounted for. However, the variance component estimate for species was significant

($p = 0.0247$), illustrating intrareceptor differences in toxicity among species as are usually observed (Clark et al., 2004). Accounting for specific sensitivities among species is critical to summarizing results from multiple toxicity studies (Anderson et al., 2008).

CLASSIFICATION PROCEDURE FOR BIOAVAILABILITY

Residuals from the most parsimonious models apportioning nuisance variation in the toxicity and bioaccumulation data sets were recovered. Relationships between model residuals and SOM, CLAY, and pH are illustrated in Figure 7. Significant ($p < 0.0001$) positive trends were associated with all three variables for the toxicity data set. Similarly, significant ($p < 0.0001$) negative trends were associated with the bioaccumulation data set. Collectively, both data sets tenably illustrate the effect of soil metal sequestration on bioavailability. Though a high degree of variation is evident in the data sets, the significant *p*-values serve to support further analysis.

A final regression tree (RT) was identified using selected soil properties exclusively. Use of internal and, in particular, external validation suggested no increase in predictive capacity beyond four terminal groups (see Figure 8). The default four-group solution, which split first on pH, then CLAY, and again on pH, is shown in Figure 9. However, subsequent bootstrap analysis suggested that SOM had substantial support for trees of MAXDEPTH 3 (50%). (Precise definitions of MAXDEPTH and other CART parameters are found in documentation of R package rpart.) Detailed examination of the results revealed that at the second split (based on CLAY), approximately the same improvement was obtained by a split on SOM. Therefore, although default results are presented, similar solutions to the final RT (see Figure 9) could use the variable SOM in some way.

The ability to use potentially important variables is limited by whether data are adequate to characterize their specific influence. In particular, certain soil properties are often observed intercorrelated; e.g., stable clay-organic matter complexes are typical (Stevenson, 1994). In fact, numerous bioavailability studies have demonstrated intercorrelation among the chemical/physical properties of their respective experimental soils (e.g., Anderson and Basta, 2009a,b; Bradham et al., 2006; Dayton et al., 2006). However, no significant intercorrelation was observed among the selected properties of the experimental soils collated in the current study, presumably due to tremendous diversity. It just so happened in these particular analyses that CLAY was selected by the CART algorithm instead of SOM at the second split, whereas bootstrap results suggest approximately the same improvement with both variables. Regardless, pH was the primary variable modifying BAF values beyond nuisance variation and is generally considered the master variable regulating metal equilibria in contaminated soil systems. Therefore, the final RT presented in Figure 9, though not uniquely supported by the available data (other solutions could be equally valid), is a simple, yet robust tool broadly applicable to the assessment of terrestrial metals bioavailability composed of exposure studies independently validated by studies on toxicity.

Central tendencies among the terminal nodes (i.e., bioavailability categories) were used to determine relative differences in bioavailability according to the proposed classification scheme. Analysis of variance of the residual values among bioavailability categories indicated highly significant ($p \leq 0.0083$) differences among all ordinal pair-wise combinations. Table 3 illustrates mean differences in residual BAF values for each category of increasing bioavailability. O*ur results suggest a maximum 3.53-fold effect of soil metal sequestration on terrestrial metals bioavailability*. When normalized to the highest category of bioavailability, relative differences equate to 70%, 41%, and 28% bioavailability for the Medium-High, Medium-Low, and Low categories, respectively (see Table 3). Overall, the validated classification procedure is proposed as a quantitative tool that broadly characterizes typical aerobic soils in terms of their potential to sequester common divalent cationic metals and mitigate their bioavailability to soil-dwelling biota.

APPLICATION TO ECOLOGICAL RISK ASSESSMENT

Although Screening Level ERAs are conservative and, hence, assume 100% bioavailability, the ecological relevance of Baseline ERAs depend entirely on the extent to which bioavailability of contaminants is accurately quantified. Direct toxicity testing of contaminated soils is the superior approach but can prohibitively add to assessment costs. If direct toxicity tests are employed, interpreting results can be operationally difficult without accounting for potential spatial patterns in bioavailability that may result from soil heterogeneity. In either case, the quantitative tools presented in the current study (see Figure 9 in conjunction with Table 3) are proposed to augment terrestrial ERAs of cationic metals contaminated soils, e.g., in support of the interpretation of toxicity testing.

Typical Baseline ERAs include site characterization, risk assessment, and risk management phases (e.g., U.S. EPA, 1998). Results of the current study can be directly applied to the site characterization and risk assessment phases. For example, in the site characterization phase, soil composition data can be included to refine Conceptual Site Models in the context of bioavailability according to the classification procedure presented in Figure 9. Based on the results of this investigation, risk assessors should, thus, routinely include relatively standard test methods for soil pH and total clay content (and SOM) in concert with total metals analyses. However, additional variables, not included in this investigation, may provide additional value-added information. Specifically, the composition of SOM and total clay content can vary greatly, which can affect soil metal sequestration (Lock and Janseen, 2001). Mineralogical analysis of clay-sized particles including amorphous oxide content can also improve on bioavailability estimates (e.g., Dayton et al., 2006; Bradl, 2004; Peijnenburg et al., 1999a). The results of this investigation are, therefore, couched as the extent to which the current literature on soil metal bioavailability could be synthesized by meta-analysis. Although a more exhaustive dataset including additional soil chemical/physical properties would likely improve the overall applicability, the results of the current study are a starting point for which additional research may improve upon pending the quantification of relevant variables in future studies.

In the risk characterization phase, Figure 9 can be used in conjunction with some categorization of total soil concentrations to develop a matrix of risk categories. For example, low concentration and low bioavailability equates to low risk. Conversely, high concentration and high bioavailability equates to high risk. Mapping heterogeneous sites by relative risk category could focus subsequent remedial efforts. Additionally, adjustment factors presented in Table 3 can be used to normalize total soil concentrations for the development of site-specific Exposure Point Concentrations (EPCs) for soil-dwelling organisms according to the classification scheme presented in Figure 9. For example, if the soil concentration of metal X has been sampled and determined to be 1,500 mg/kg, and the soil has a pH greater than 4.7 but less than 6.5 and clay content less than 26%, the bioavailability category (see Figure 9) would be Medium-High, leading to a factor difference of 1.42 (or 70% bioavailable) relative to the High category (see Table 3), which would result in a bioavailability-adjusted EPC of 1,050 mg/kg ($1,500 \times 0.70$). Alternatively, if the soil concentration of metal X has been sampled and determined to be 1,500 mg/kg, and the soil has a pH greater than 4.7 and clay content greater than 26%, the bioavailability category (see Figure 9) would be Low, leading to a factor difference of 3.53 (or 28% bioavailable) relative to the High category (see Table 3), which would result in a bioavailability-adjusted EPC of 420 mg/kg (1,500 × 0.28). The adjustment factors in Table 3 are calculated relative to the highest-bioavailability category. The approach does not assume that the highest bioavailability values in the data would be used in all situations. Bioavailability-adjusted EPCs for soil-dwelling organisms can be used as input to trophic transfer models to predict site-specific exposures for higher-order wildlife species explicitly applying soil bioavailability concepts to risk estimates for the relevant receptor(s) evaluated at a given site.

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Table 1. **Summary of Data Used in Meta-analyses**

Data set	Reference	Number of Soils	Range in soil properties		
			pH[a]	Clay (%)	SOM (%)[b]
Bioaccumulation	(Yanai et al., 2006)	25	4.30−7.77	1.8−44	2.40−29.2
	(Dayton et al., 2006)	21	4.00−7.90	10−60	0.400−5.80
	(Bradham et al., 2006)	21	4.00−7.90	10−60	0.400−5.80
	(Peijnenburg et al., 1999a)	20	3.81−7.43	0.20−47	0.300−15.1
	(Peijnenburg et al., 1999b)	20	3.81−7.43	0.20−47	0.300−15.1
	(Janssen et al., 1997)	20	3.81−7.43	0.20−47	0.300−15.1
	(Vijver et al., 2001)	16	3.09−7.30	0.20−47	0.300−35.0
	(Peijnenburg et al., 2000)	15	4.36−7.22	1.3−47	0.900−23.4
	(Nahmani et al., 2009)	7	4.58−6.54	1.1−5.0	0.284−2.36
	(Anderson and Basta, 2009a)	5	3.67−7.34	6.8−42	0.810−4.78
	(Weng et al., 2003)	4	4.70−6.80	4.0−4.0	4.00−4.00
	(Smilde et al., 1992)	3	4.20−7.20	3.0−40	3.70−7.00
	(Elmosly and Abdel-Sabour, 1997)	3	7.40−8.10	5.5−20	0.0500−1.00
	(Bjerre and Schierup, 1985)	3	6.20−7.50	4.9−8.0	1.90−17.7
Toxicity	(Oorts et al., 2006)	35	3.00−7.70	1.0−55	0.500−66.1
	(Criel et al., 2008)	19	3.00−7.50	5.0−51	0.800−46.6
	(Rooney et al., 2006)	18	3.40−7.50	5.0−51	0.760−46.6
	(Rooney et al., 2007)	16	3.60−7.70	0.40−55	0.500−66.1
	(Smolders et al., 2004)	12	3.00−7.50	5.0−51	0.800−46.6

Table 1. Summary of Data Used in Meta-analyses (continued)

Data set	Reference	Number of Soils	Range in soil properties		
			pH[a]	Clay (%)	SOM (%)[b]
Toxicity cont.	(Li et al., 2009)	10	4.30−7.53	1.0−48	1.60−10.6
	(Spurgeon and Hopkin, 1996)	9	4.00−6.00	20−20	5.00−15.0
	(de Haan et al., 1985)	6	4.60−5.60	4.0−58	1.60−19.4
	(Anderson and Basta, 2009b)	5	3.67−7.34	6.8−42	0.812−4.78
	(Vonk et al., 1996)	5	3.50−6.80	1.9−20	2.40−10.0
	(Donkin and Dusenbery, 1994)	4	5.10−6.20	16−39	1.70−3.40
	(Weng et al., 2003)	4	4.70−6.80	4.0−4.0	4.00−4.00
	(Donkin and Dusenbery, 1993)	4	5.10−6.20	16−39	1.70−3.40
	(ESG International Inc. and Aquaterra Environmental Consulting, 2000)	3	6.05−8.10	11−30	2.90−12.8
	(Reber, 1989)	3	5.60−7.00	3.2−21	1.67−2.62
	(Sheppard et al., 1993)	2	7.30−7.90	43−46	2.70−8.90
	(Gunther and Pestemer, 1990)	1	6.10	9.9	1.31
	(Korthals et al., 1996)	1	4.10	4.0	1.90
	(Ma, 1982)	1	7.30	17	8.00
	(van Gestel and van Dis, 1988)	1	7.00	4.3	1.70
	(Spurgeon et al., 2000)	1	6.35	9.7	2.35
	(Kjaer and Elmegaard, 1996)	1	6.40	11	3.40

Table 1. Summary of Data Used in Meta-analyses (continued)

Data set	Reference	Number of Soils	Range in soil properties		
			pH[a]	Clay (%)	SOM (%)[b]
Toxicity cont.	(Hague and Ebing, 1983)	1	6.10	3.2	1.00
	(Dang et al., 1990)	1	8.30	24	0.560
	(TN & Associates Inc., 2000)	1	6.32	3.2	0.200
	(Pedersen et al., 2000)	1	6.70	14	9.00
	(Kapustka et al., 2006)	1	6.32	3.2	0.100
	(Howcroft et al., 2009)	1	5.60	13	4.72

[a] pH values include KCl and $CaCl_2$ extracts.
[b] Studies that only reported organic C values were doubled to convert to soil organic matter (SOM) values (Sleutel et al., 2007).

Table 2. Variable Selection Results[a] from Apportionment Analysis for Both Bioaccumulation and Toxicity Data Sets

Data Set	Variables	K[b]	$-2(\ell_{max}$[c]$)$	AICc[d]	ΔAICc[e]	w[f]
Toxicity	METAL + RECEPTOR + ENDPOINT + PARAMETER	27	3,785.9	3,841.1	0.0	0.91
	METAL + ENDPOINT(RECEPTOR) + PARAMETER	31	3,782.2	3,845.8	4.7	0.09
	METAL + ENDPOINT + PARAMETER	26	3,797.6	3,850.7	9.6	0.01
Bioaccumulation	METAL + ENDPOINT + DOSE + METAL × DOSE	31	1,580.7	1,646.8	0.0	0.91
	METAL + DOSE + METAL × DOSE	29	1,591.5	1,653.1	6.3	0.04
	METAL + TYPE + DOSE + METAL × DOSE	30	1,589.5	1,653.4	6.5	0.03
	METAL + RECEPTOR + DOSE + METAL × DOSE	30	1,591.4	1,655.3	8.4	0.01

[a] Only those models with a cumulative total of Akaike weights >99% are presented.
[b] Number of model parameters.
[c] Maximum *Log-likelihood*.
[d] Small-sample adjusted Akaike Information Criterion (AICc).
[e] AICc difference.
[f] AICc weight.

Table 3. Proposed Adjustments to Total Soil Concentrations That Account for Metals Bioavailability Based on the Central Tendencies of the Terminal Nodes (i.e., Bioavailability Categories) from Figure 9. (See Example)

	Bioavailability Category			
	High	**Medium-High**	**Medium-Low**	**Low**
Factor difference	1.00	**1.42**	2.44	3.53
Percent bioavailable	100	**70**	41	28

Example: The value of 1.42 for the medium-high category can be obtained in two steps from results displayed in Figure 9: First apply the antilogarithm (exponential) to the mean values for "H" and "M-H" groups, which were calculated in the natural logarithm scale. Thus we compute values 1.878 (H) and 1.323 (M-H). Finally, the value displayed is 1.42 = 1.878/1.323. The corresponding percent bioavailable is the inverse of this value × 100: 70 = 100 × (1/1.42).

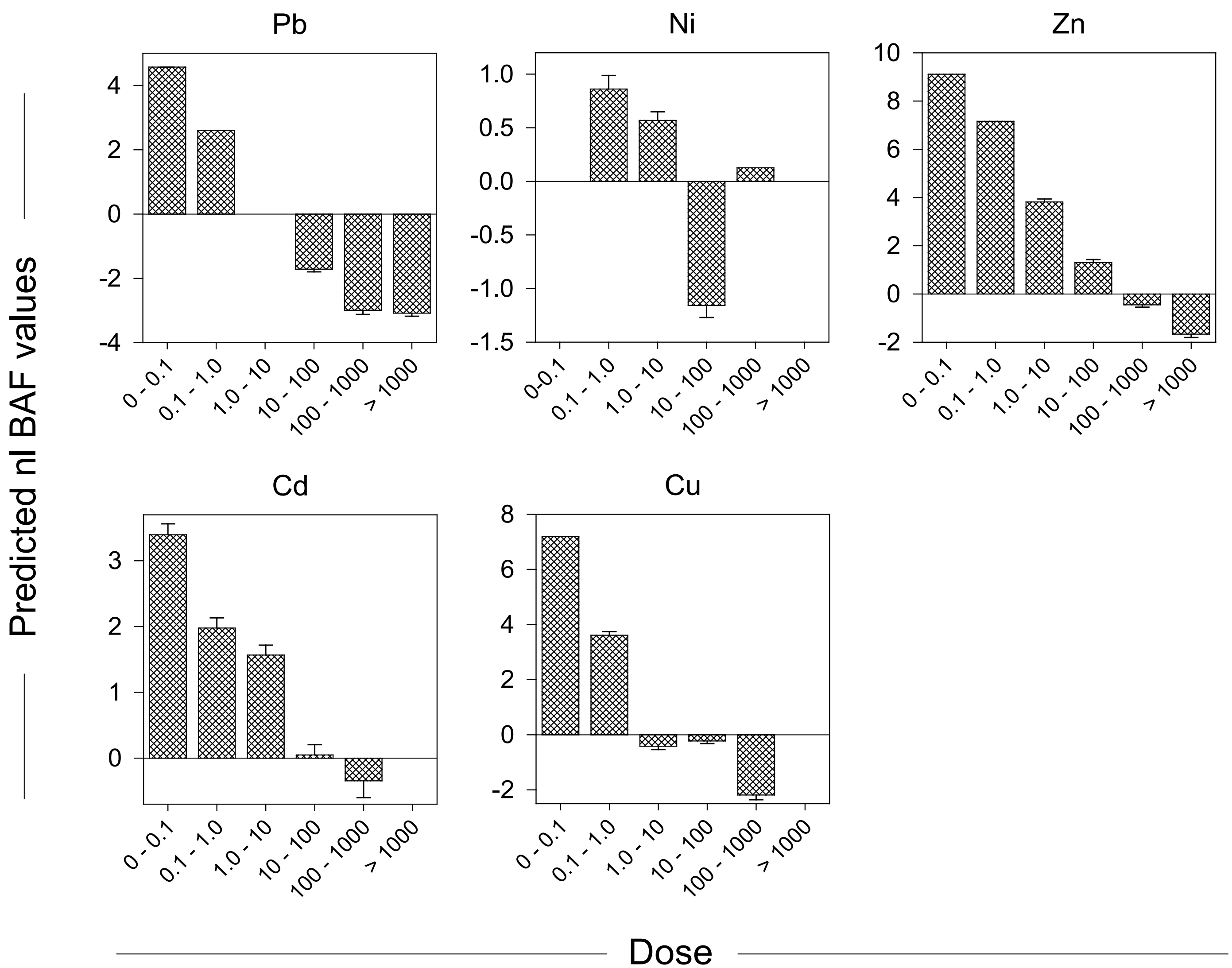

Figure 1. Mean (± 1 SE) Predicted Natural Log (nl) BAF Values by Dose, as Stratified by Metal, from the Most Parsimonious Bioaccumulation Model Apportioning Nuisance Variation.

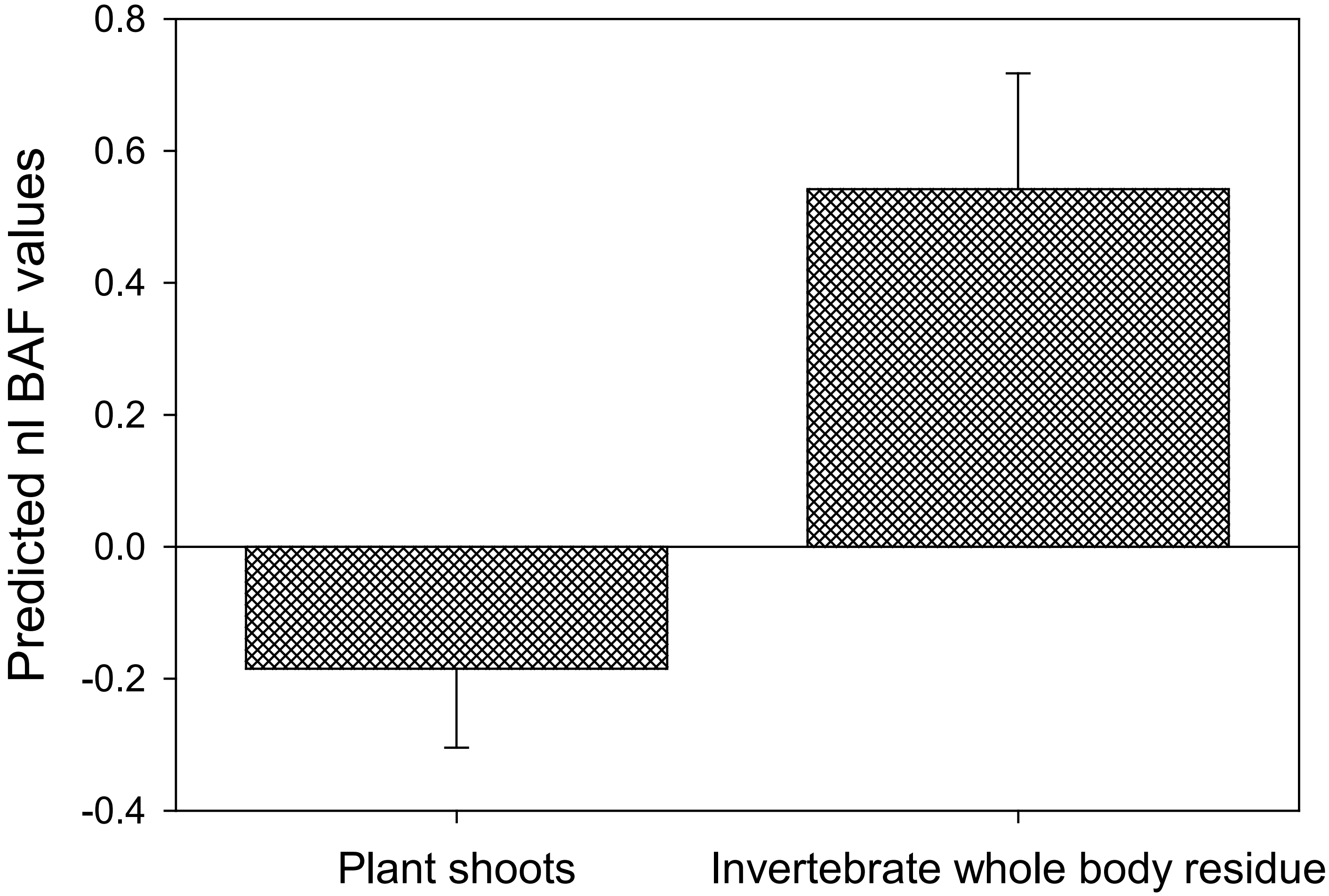

Figure 2. Mean (± 1 SE) Predicted Natural Log (nl) BAF Values by Endpoint (confounded by receptor) from the Most Parsimonious Bioaccumulation Model Apportioning Nuisance Variation.

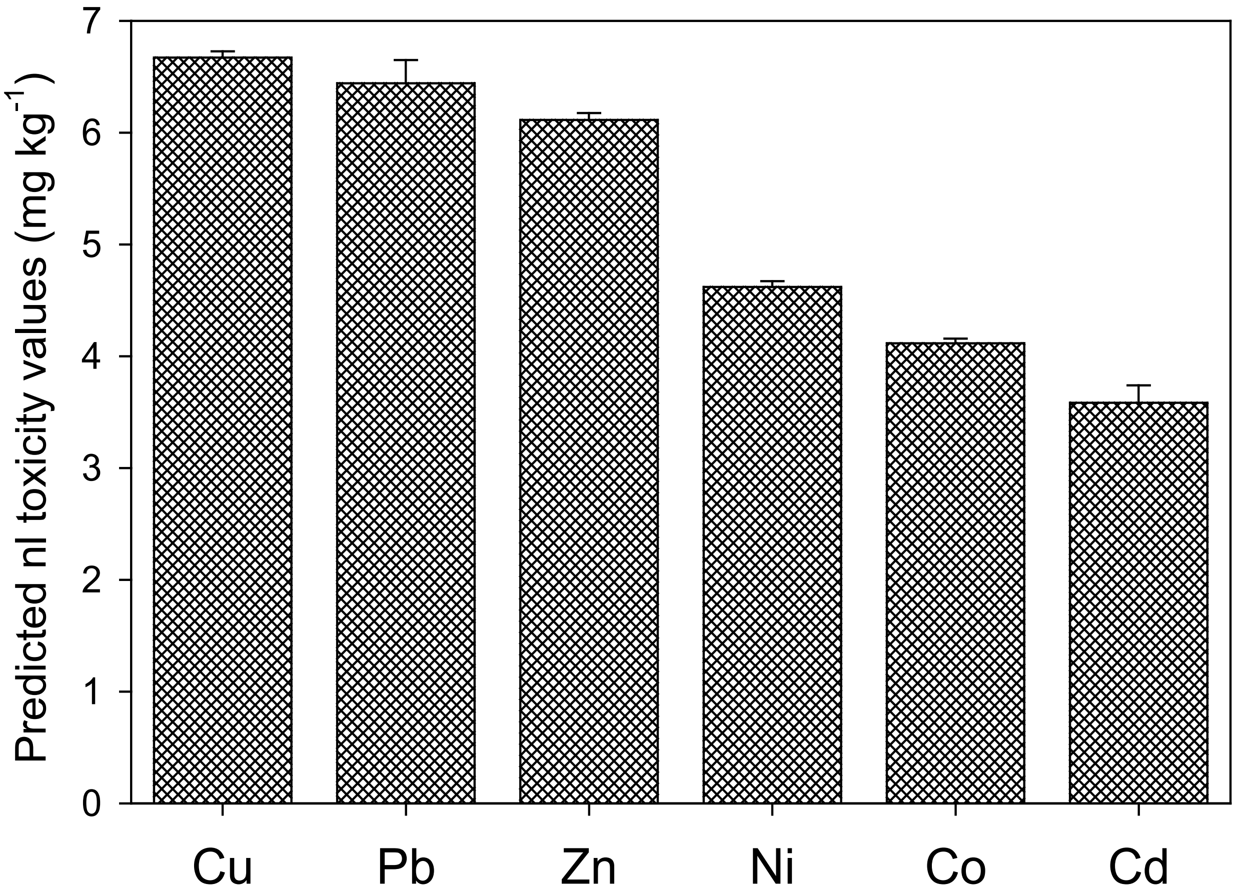

Figure 3. Mean (± 1 SE) Predicted Natural Log (nl) Toxicity Values by Metal from the Most Parsimonious Toxicity Model Apportioning Nuisance Variation.

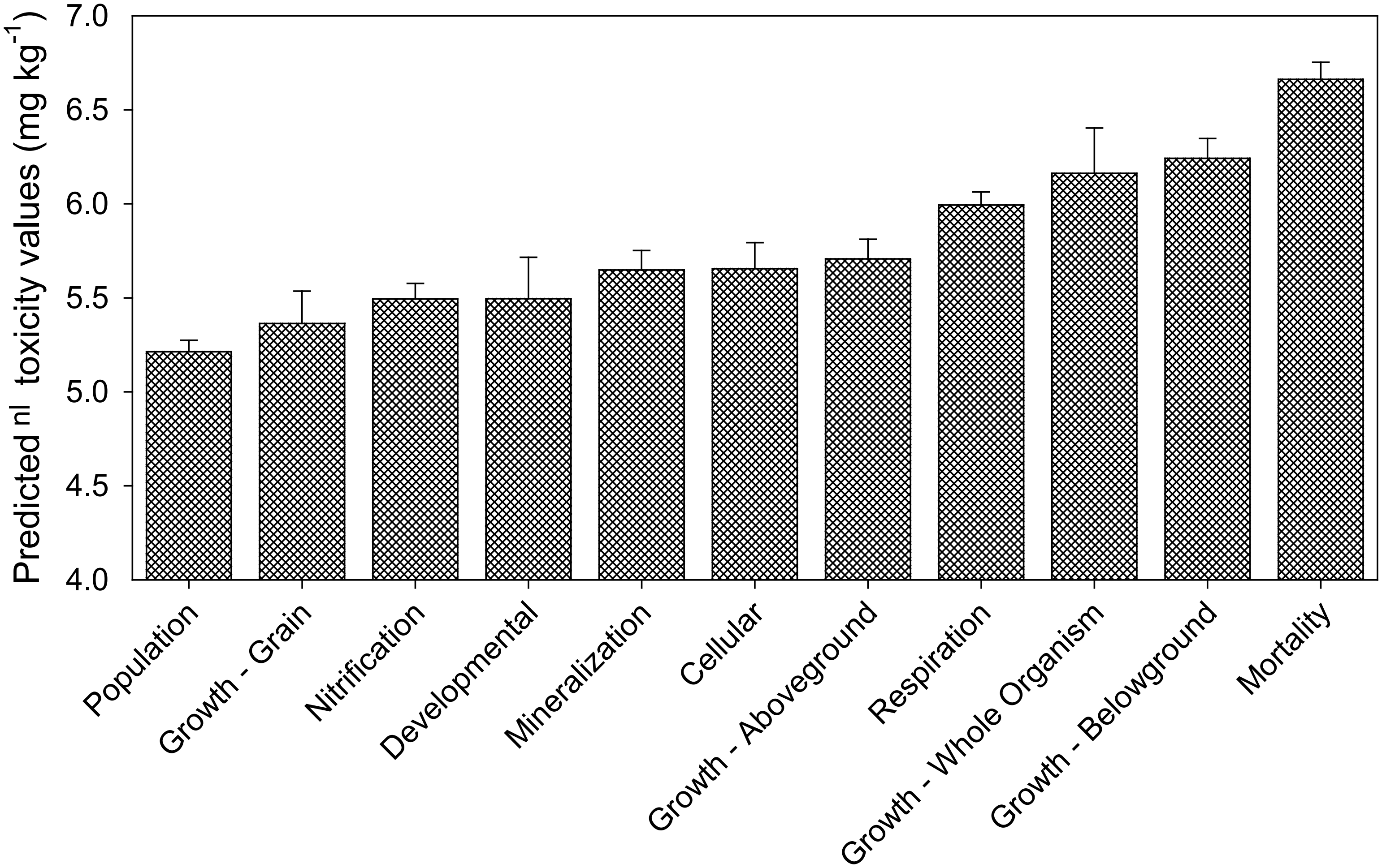

Figure 4. Mean (± 1 SE) Predicted Natural Log (nl) Toxicity Values by Endpoint from the Most Parsimonious Toxicity Model Apportioning Nuisance Variation.

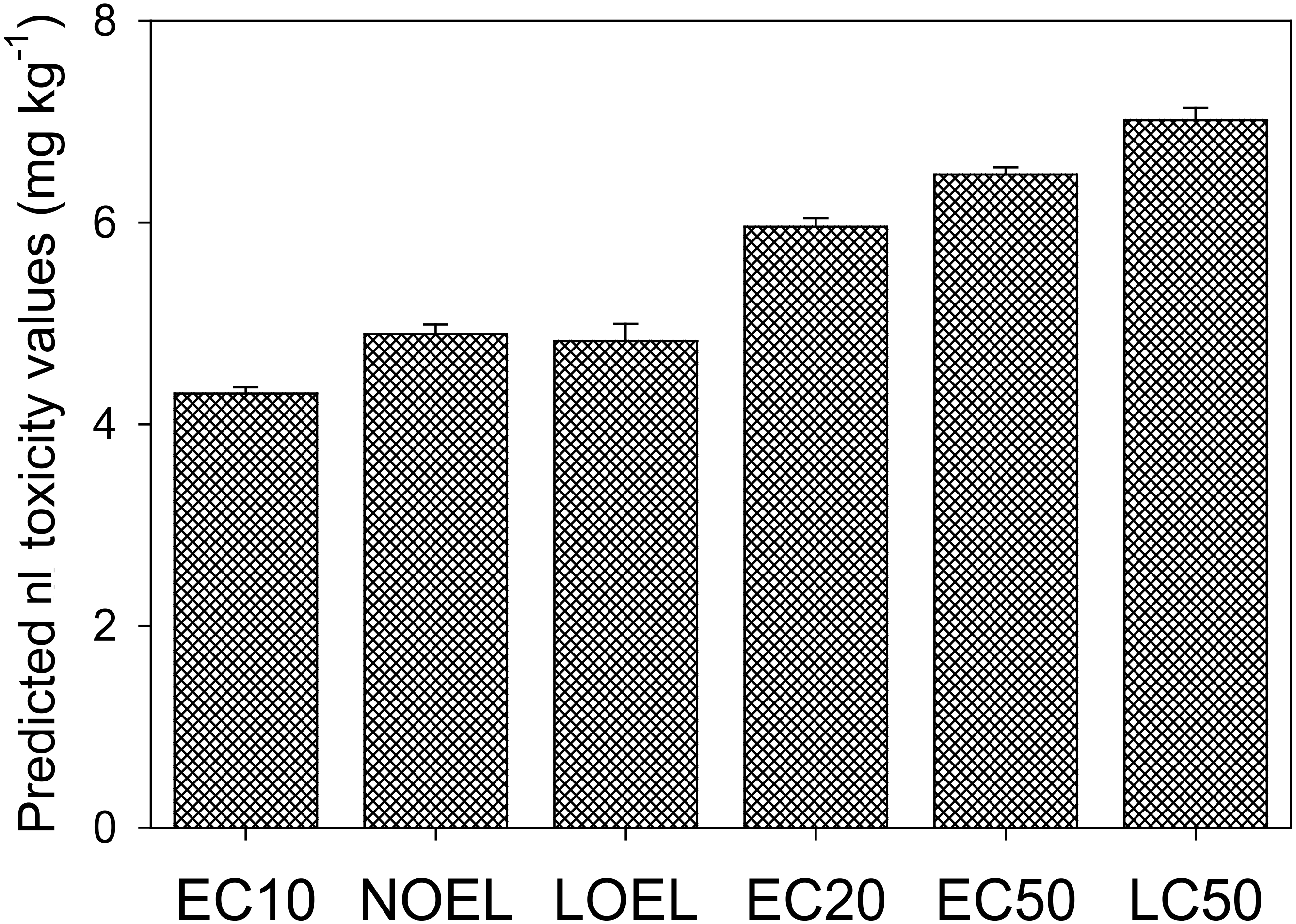

Figure 5. Mean (± 1 SE) Predicted Natural Log (nl) Toxicity Values by Parameter from the Most Parsimonious Toxicity Model Apportioning Nuisance Variation.

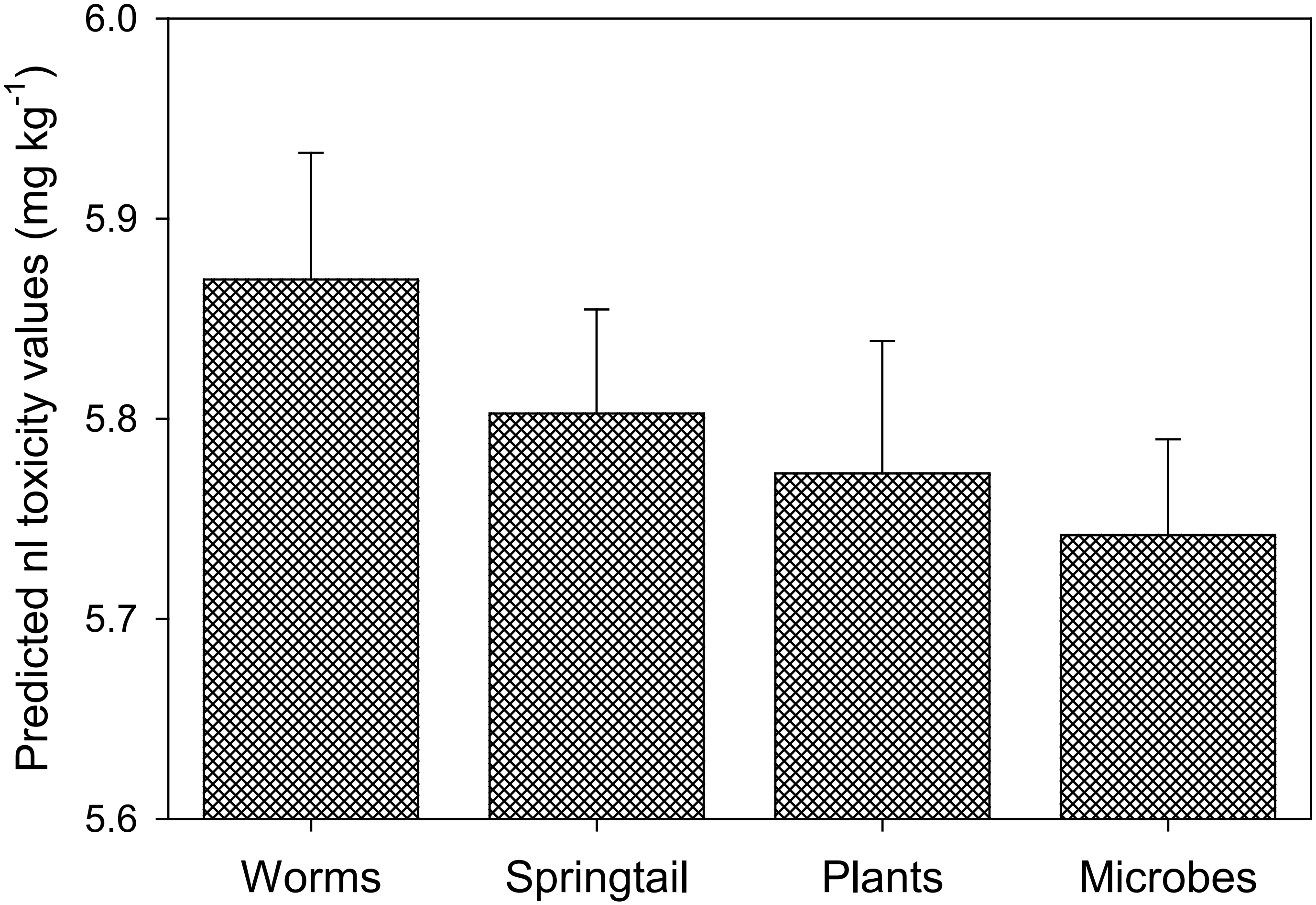

Figure 6. Mean (± 1 SE) Predicted Natural Log (nl) Toxicity Values by Receptor from the Most Parsimonious Toxicity Model Apportioning Nuisance Variation.

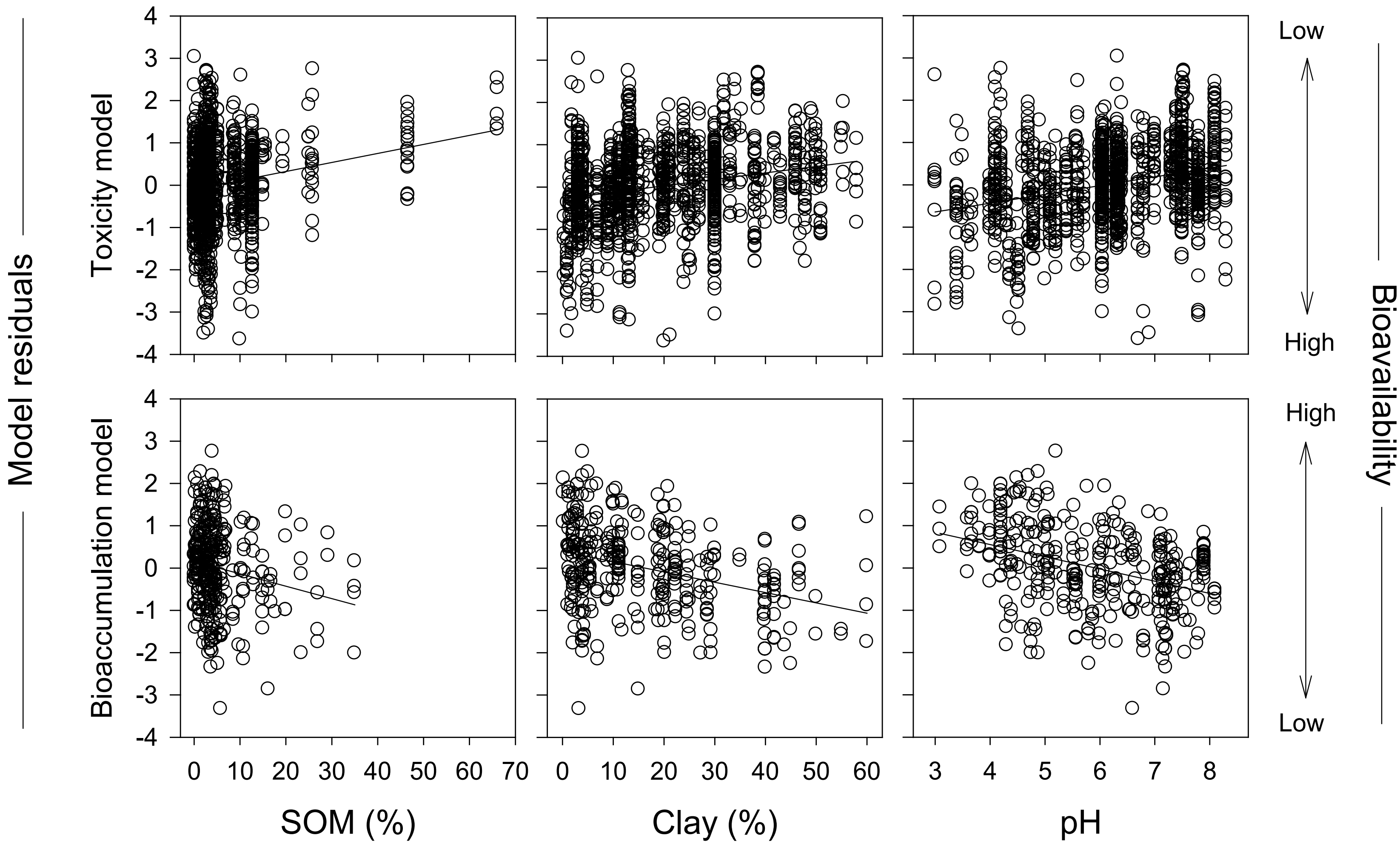

Figure 7. Relationships Between Model Residuals and Soil Organic Matter (SOM), Clay Content, and pH for the Most Parsimonious Toxicity and Bioaccumulation Linear Models Apportioning Nuisance Variation (see Table 2).

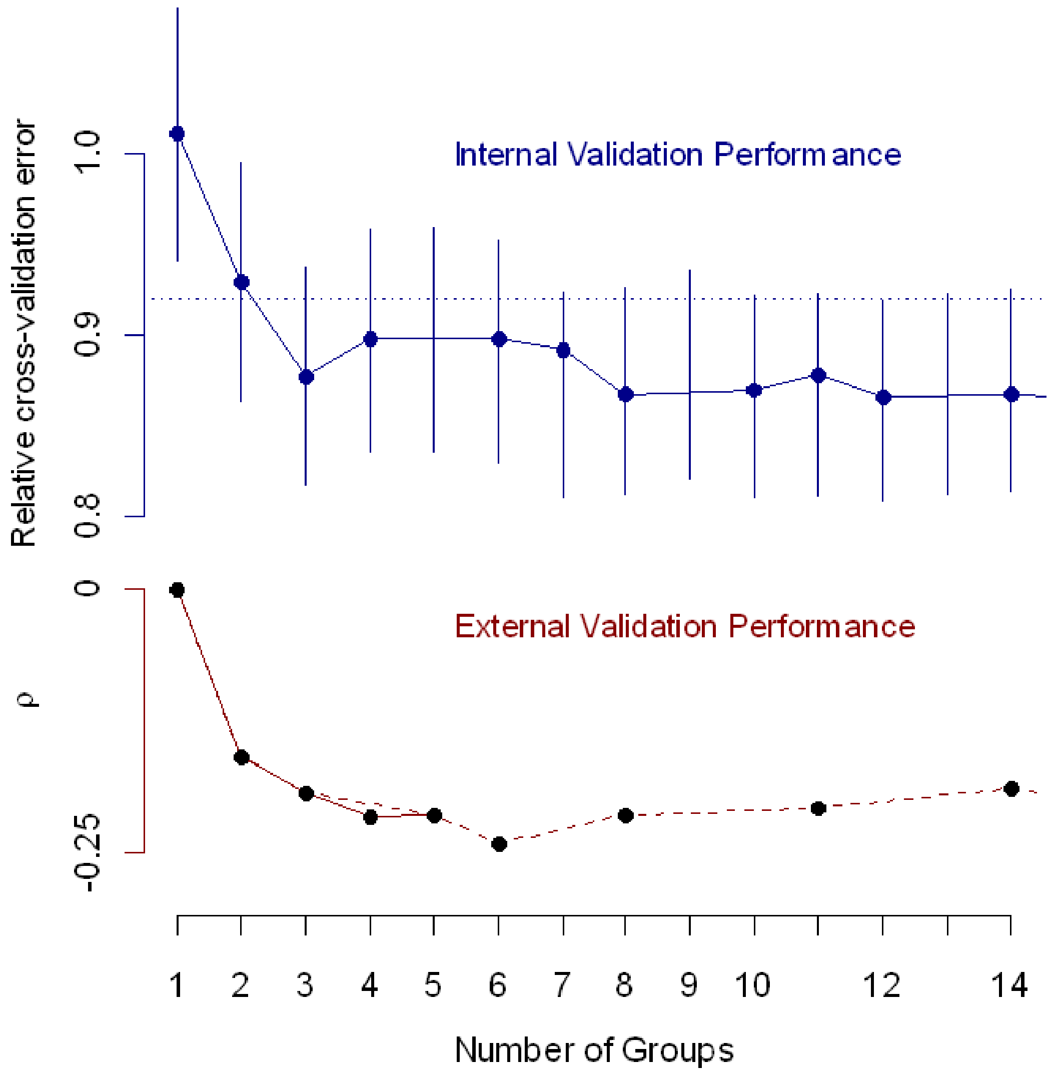

Figure 8. Internal (Bioaccumulation Data Set) and External (Toxicity Data Set) Validation Results of Cross-Validation Analyses Used to Determine the Optimal-Sized Regression Tree. The upper figure relates the sum of squared error (from *k*-fold cross validation) for an RT model, relative to the error from use of an RT with no splits (i.e., use of the simple grand mean), to the number of terminal groups. The lower figure relates the Spearman correlation between predictions and toxicity values to the number of groups.

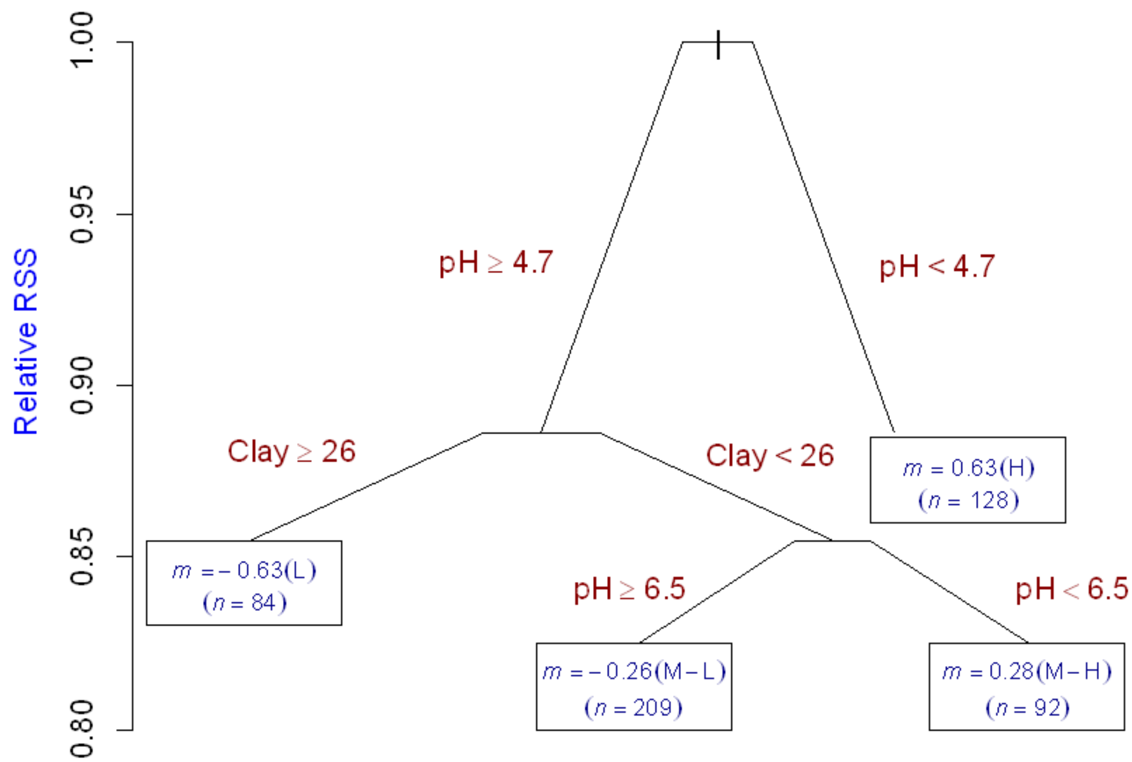

Figure 9. Regression Tree Results Constrained to Four Terminal Nodes (see Figure 8). Mean (*m*) and sample size (*n*) values are characterized for each terminal node, which are classified into bioavailability categories of High (H), Medium-High (M-H), Medium-Low (M-L), and Low (L). Relative RSS gives the sum of squared error for an RT with a given number of splits, relative to an RT with no splits (i.e., simple use of the grand mean).