EFFECT OF SOIL PROPERTIES ON THE BIOAVAILABILITY AND TOXICITY OF METALS TO *EISENIA ANDREI*

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Submitted to the Faculty of
the Graduate College of
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2002

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METALS TO *EISENIA ANDREI*

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ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my advisors, Dr. Roman Lanno and Dr. Nicholas Basta, for their scientific and professional guidance, encouragement, support, and friendship. My sincere appreciation extends to my committee members, Dr. Mark Payton and Dr. David Janz, whose guidance, assistance, encouragement, and friendship are also invaluable. I wish to thank my Master's degree mentor, Dr. Roger Bacon, for his steadfast belief in my abilities. Many thanks to my fellow graduate students for their assistance and support. I wish to thank Elaine Stebler for her support and assistance with the earthworms.

I would like to thank my family for their love and encouragement; I could not have pursued this academic adventure without them. I would also like to thank Lynne Junker for her friendship and constant encouragement and enthusiasm that continues to amaze and enlighten me. Last, but not least, I would like to give my special appreciation to Derrick Chappell for his love and understanding through this process, and for his friendship and unwavering sense of calmness. His assistance with this project and the bioassays was invaluable.

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NOMENCLATURE

As	arsenic
BSAF	biota-soil accumulation factor
$BSAF_{Ca(NO3)2}$	biota-soil accumulation factor based on calcium nitrate extractable soil concentration
$BSAF_{PW}$	biota-soil accumulation factor based on pore water extractable soil concentration
$BSAF_{Total}$	biota-soil accumulation factor based on total soil concentration
CI	confidence interval
$Ca(NO_3)_2$	calcium nitrate
Cd	cadmium
HCl	hydrochloric acid
HNO_3	nitric acid
LC_{50}	median lethal concentration
OC	organic carbon
pH	$-\log_{10}$ [hydrogen ion activity]
Pb	lead
USEPA	United States Environmental Protection Agency
Zn	zinc

INTRODUCTION

This study was conducted to better understand the effect of soil properties on the bioavailability and toxicity of As, Cd, Pb, and Zn, to earthworms. In order to protect and/or restore soil ecosystems, it is necessary to accurately characterize the risk posed to soil organisms by metals and other contaminants. Risk is directly related to metal bioavailability, which depends upon the metal concentration, the geochemical forms of metal, the species of organism exposed, physical and chemical characteristics of the exposure environment, and the exposure duration. There are direct and indirect methods for determining bioavailability. Direct measures of bioavailability incorporate organismal responses and/or internal chemical concentrations to estimate bioavailability. Indirect measurements of bioavailability do not use living organisms to estimate the bioavailability of chemicals from soil. Instead, they utilize measured concentrations of chemical species that are potentially available for uptake. However, it is necessary to integrate chemical (indirect) and biological (direct) measures to accurately reflect the bioavailability of metals in soil and to protect ecological receptors. The objective of this study was to examine the effect of soil properties (e.g., pH, organic matter content, clay content), on metal bioavailability and toxicity in earthworms (*Eisenia andrei*) and develop a mathematical model to describe this relationship.

This document consists of four chapters, each reporting studies conducted during my Ph.D. program. Each chapter is presented in formats suitable for publication in professional journals.

CHAPTER 1

EFFECT OF SOIL PROPERTIES ON THE BIOAVAILABILITY AND TOXICITY OF LEAD TO *EISENIA ANDREI*

ABSTRACT

Pb bioavailability and toxicity is directly influenced by soil properties. In the present study, the relationship between soil properties, and Pb bioavailability and toxicity in earthworms (*Eisenia andrei*) exposed to 22 field soils spiked with Pb is investigated to develop a mathematical model to describe this relationship. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to the same total Pb concentration in spiked soils. Statistical models were developed for earthworm mortality, cocoon production, internal concentrations, extractable Pb levels, and bioaccumulation factors. Soil pH was found to be the most important soil property modulating Pb bioavailability for mortality, internal concentrations, bioaccumulation factors, and extractable Pb levels ($P < 0.05$). Regression analysis results established a relationship ($r^2 = 0.64$) for 0.5 M $Ca(NO_3)_2$-extractable Pb and internal Pb concentrations ($P < 0.01$). The 100-fold difference of $Ca(NO_3)_2$-extractable Pb and complete mortality in soils with > 900-mg kg^{-1} $Ca(NO_3)_2$-extractable Pb are evidence of the effects of soil properties modifying environmentally available Pb. In soils with the largest bioaccumulation factors, little or no mortality was observed suggesting that bioaccumulation factors are poor indicators of Pb toxicity to earthworms.

INTRODUCTION

Lead (Pb) contamination of soil is a worldwide problem that may pose a risk to soil organisms. Sources of Pb contamination of soil include mining and smelter operations, industrial discharge, coal, gasoline, Pb paint, and sewage sludge (Adriano, 2001). In order to protect and/or restore soil ecosystems, it is necessary to accurately characterize the risk posed to soil organisms by Pb and other contaminants. Risk is directly related to Pb bioavailability, which depends upon Pb concentration, the geochemical forms of Pb, the species of organism exposed, physical and chemical characteristics of the exposure environment, and the exposure duration. Bioavailability and toxicity are not permanent properties of soil but vary with the interaction between the soil and the organism (Lanno and McCarty, 1997). There are direct and indirect methods for determining bioavailability. Direct measures of bioavailability incorporate organismal responses and/or internal chemical concentrations to estimate bioavailability. Indirect measurements of bioavailability do not use living organisms to estimate the bioavailability of chemicals from soil. Instead, they utilize measured concentrations of chemical species that are potentially available for uptake. Weakly bound or available metals are believed to be available for uptake by earthworms (Posthuma et al., 1997; Peijnenburg et al., 1999b). The authors suggested field soils should be tested to further validate the use of weak electrolyte extractions as surrogate measures of bioavailability. Only an organism can determine bioavailability or toxicity (Lanno and McCarty, 1997). However, it is necessary to integrate chemical (indirect) and biological (direct)

measures to accurately reflect the bioavailability of Pb in soils and to protect ecological receptors.

Soil protection guidelines based on total Pb levels have been suggested for the protection of ecological receptors (USEPA, 2000). Due to soil modifying factors, total Pb concentrations are inaccurate for predicting soil organism toxicity (Ma, 1982; Beyer at al., 1987; Morgan and Morgan, 1988; McLean and Bledsoe, 1992). Risk to soil organisms based on total contaminant concentration is not an accurate predictor of adverse effects (Peijnenburg et al., 1999a) because exposure expressed as total Pb does not consider the effects of soil modifying factors on Pb bioavailability. As an example, soil pH is inversely related to Pb solubility and availability (McBride, 1989; Rieuwerts et al., 1998). Earthworms accumulate Pb more readily in soils with decreased pH and organic matter than in soils with increased pH and organic matter (Edelman et al., 1983). Due to modifying factors, soil metal is often less than 100% available for uptake by organisms (Conder and Lanno, 2000). The objective of this study was to examine the relationship between soil properties (e.g., pH, organic matter content, clay content) and Pb bioavailability and toxicity in earthworms (*Eisenia andrei*) and develop a mathematical model to describe this relationship.

METHODS AND MATERIALS

Soil collection and preparation were conducted using standard soil testing methods (See Schroder et al., in preparation, for complete methods). Twenty-

two soils with diverse paleoclimatology and geology were collected from Oklahoma and central Iowa to obtain Mollisols with a high organic C content. Soils were spiked with reagent grade $Pb(NO_3)_2$ to obtain soil concentrations of approximately 2,000 mg Pb kg^{-1}. Spiked soils were subjected to four wet-dry cycles (see Schroder et al., in preparation, for more detail) to achieve adequate reaction with the soil matrix and reduce the "salt effect" where heavy metal availability is greater in spiked soil than aged contaminated soil with similar metal contamination (Logan and Chaney, 1983). Total lead in collected soils was determined by an acid digestion microwave technique according to U.S. EPA Method 3051 for confirmation of background Pb concentrations prior to analysis of chemical and physical properties (U.S. EPA 1994). Soil pH was determined in 1:1 soil:water suspension (Thomas, 1996). Soil organic C content was determined by acid dichromate digestion according to Heanes (1984). Cation exchange capacity of non-calcareous soil (soil pH < 7.0) was determined using a procedure adopted from Hendershot and Duquette (1986). Cation exchange capacity of calcareous soils (soil pH > 7.0) was determined according to the method of Polemio and Rhoades (1977). Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). $Ca(NO_3)_2$-extractable Pb was determined by placing soil (1.0 g) in a 50 mL centrifuge tube, extracting with 20.0 mL of 0.5 *M* $Ca(NO_3)_2$ solution, and shaking tubes on a reciprocal shaker for 16 h. The solutions were then centrifuged at 10,000 rpm for 15 min, filtered through a 0.45 μm membrane filter, acidified with 1.0 mL of trace metal concentrated HCl, and stored at 4 °C until analysis of metal by ICP-AES. Spiked soils were

digested by microwave according to U.S. EPA Method 3051 to determine total lead concentrations. Blanks, spikes and certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were digested and analyzed for quality assurance and quality control in the determination of metal content in soil.

Earthworm bioassays

Twenty-eight day bioassays using *Eisenia andrei* were conducted with field soils spiked with 2,000 mg Pb kg^{-1}. The bioassays were performed in triplicate for each soil-Pb combination and conducted using mature (clitellate) manure worms (*E. andrei*) according to a standard protocol (American Society for Testing and Materials, 1997). The 200-g soil samples were moistened and maintained between 1/3 bar and saturation, placed in glass jars with 3 small air holes in the lid, and acclimated in an environmental chamber maintained at 20±1°C for 24 h prior to the addition of 10 earthworms per replicate. Twenty-four hours prior to the addition of earthworms to test soils, mature (clitellate) earthworms weighing approximately 0.2-0.4 g were removed from synchronized in-house cultures, rinsed with reagent grade water, and placed on moist filter paper for 24 hours to depurate most of the bedding material from their intestinal tracts (Van Gestel et al. 1993). At the start of the toxicity test, randomly chosen earthworms were removed from the filter paper, rinsed, and separated into replicates of 10 earthworms. Each replicate was blotted dry, weighed, and transferred to one of three jars prepared for each soil. Testing was conducted in an environmental chamber maintained at 20 ± 1°C with constant light. Earthworms were monitored

after six hours for physical condition and to determine if burrowing had occurred. Earthworms were observed daily for the first eight days and three times a week thereafter for the remainder of the test to assess the general condition of the worms and remove mortalities. Cocoons were collected on a daily basis by hand sorting. Simultaneously, observations on earthworm performance in ASTM artificial soil (American Society for Testing and Materials, 1997) and unspiked reference soils from each site served as controls for quality assurance with respect to survival, cocoon production, and growth. Artificial soils consisted of 69.5% silica sand, 20% kaolin clay, 10% 2-mm sieved *Sphagnum* peat moss, and approximately 0.5% $CaCO_3$ added to adjust the pH to 7.0±0.5. Reagent grade water was added to hydrate the artificial soil to 45% of its dry weight. All soil materials used were hydrated and allowed to acclimate in the environmental chamber maintained at 20±1°C 24 hours prior to the start of the tests. Earthworms were judged dead if no response was observed after gentle stimulation with a blunt probe. Dead earthworms were removed, rinsed thoroughly with reagent grade water, individually wrapped in aluminum foil, and frozen at -20°C for subsequent analysis. At day 28 of each study, live earthworms were depurated for 24 hours on moist filter paper, rinsed, weighed, and stored as described above. Upon the completion of toxicity tests, individual soil replicates from all experiments were stored at -20°C in Ziploc® freezer bags.

Internal concentrations

Earthworm Pb concentrations were determined as described by Morgan et al. (1982). An individual worm from each replicate (3 replicates per soil-Pb combination) was removed from the freezer, dried for 24 hours at 105°C in a pre-weighed 10 mL glass beaker, and weighed. Individual worms were then wet digested using 5 mL concentrated trace metal grade HNO_3 (Fisher Scientific). Digests were evaporated to dryness, resolubilized in 3 mL 0.5 M HNO_3, heated for 15 minutes at 60°C, and diluted to final volume of 10 mL with 0.5 M HNO_3. Worm digests were stored in Nalgene® low-density polyethylene bottles until analysis. Pb concentrations in digests were measured using graphite furnace AAS (HGA PerkinElmer Analyst 700). The limit of detection for Pb in earthworm tissue digests was 0.4 μg/L. Pb concentrations in worm tissues were expressed on an mg kg^{-1} dry weight basis. All analyses included procedural blanks, spikes, and certified reference material (lobster hepatopancreas, TORT-2, National Research Council, Canada). Mean (%RSD) spike and certified reference material recoveries were 97 (2.8%) and 98 (1.2%), respectively.

Data analysis

Statistical analyses were performed using PC SAS Version 8.2 (SAS Institute Inc., Cary, NC). LC_{50} values were based on models produced by Proc Probit. Empirical models were developed for comparison to models in the literature. Backwards-stepwise regression analysis was used to derive empirical models

capable of predicting effects of Pb on earthworm mortality, internal concentrations, and cocoon production based on soil properties. The backwards-stepwise regression analysis was used to identify critical soil properties explaining most of the variation. Soil properties that did not explain a significant part of the variation ($P > 0.10$) were not used as independent variables in the multiple regression equation. Statistical models capable of predicting effects of Pb bioavailability, based on soil properties were obtained from the regression analysis. The multiple regression functions have the format:

$$Y = b_0 + b_1(pH) + b_2(clay) + b_3(OC)$$

Where Y = extractable Pb, earthworm response (mortality, internal concentrations, and cocoon production), or biota-soil accumulation factors, A, B, and C = soil properties (pH, clay, OC), and a, b, and c = coefficients.

Empirical models were compared with quantitative causal values for each soil property provided by path analysis models. Path analysis, an extension of the regression model, is a statistical technique that differentiates between correlation and causation (Basta et al., 1993). Path analysis was used to decompose correlations in the model into direct or causal effects of soil properties (Loehlin, 1987) on earthworm mortality, internal concentrations, and cocoon production. Path analysis allows the partitioning of simple correlation coefficients between dependent (e.g. mortality) and independent variables (soil properties) into direct and indirect effects (Basta et al., 1993). Path analysis also provides a numerical value for each direct and indirect effect and indicates the relative strength of that

correlation or causal influence (Loehlin, 1987). Direct effects are standardized partial regression coefficients designated as path coefficients (Basta et al., 1993). Direct and indirect effects are derived from multiple linear regression of soil properties on earthworm response (mortality, internal concentrations, and cocoon production) and simple correlation values between soil properties. In addition, an uncorrelated residual (U) was determined from this model using the following equation:

$$U = \sqrt{1 - R^2}$$

A path analysis model was composed to study the effect of pH, OC, and clay on earthworm mortality (Figure 1). Direct effects (path coefficients) of soil properties on earthworm mortality are represented by the single-headed arrows while the double-headed arrows represent intercorrelation coefficients. Indirect effects of soil properties on earthworm mortality are determined from the product of one double-headed arrow and one single-headed arrow (Basta et al., 1993). Path analysis results were derived using the following equations (Williams et al., 1990):

$$r_{14} = P_{14} + r_{12}P_{24} + r_{13}P_{34} \qquad [1]$$

$$r_{24} = r_{12}P_{14} + P_{24} + r_{23}P_{34} \qquad [2]$$

$$r_{34} = r_{13}P_{14} + r_{23}P_{24} + P_{34} \qquad [3]$$

where r_{i4} corresponds to the simple correlation coefficient between the soil property and earthworm response, P_{i4} are path coefficients (direct effects) of soil property i on earthworm response, and $r_{ij}P_{i4}$ are the indirect effects of soil

property j through property i on earthworm response. Subscript designations are: (1) pH, (2) OC, (3) Clay, and (4) earthworm response.

The path analysis results can be presented in a concise table (Williams et al., 1990). This table provides underlined diagonal numbers indicating direct effects and off-diagonal numbers indicating indirect effects. The position of each response in the table corresponds to its position in the matrix of respective equations (equations [1], [2], [3], above). This format allows all potential tables to be presented as one table.

RESULTS AND DISCUSSION

Metal availability

The 22 soils collected had a wide range of soil properties including soil pH (4.0–8.0), cation exchange capacity (3.0 to 32.4 $cmol_c\ kg^{-1}$), organic C (0.3 to 3.0%), and clay content (5.0 to 71%) (Table 1). The Pb content of collected soils was similar to uncontaminated background soil contents prior to Pb amendment (See Schroder et al., in preparation). The target value for Pb amended in the test soils was 2,000 mg Pb kg^{-1}, based upon earthworm responses in range-finder tests. The mean total Pb content in test soils of 1880 mg Pb kg^{-1}, slightly lower than the target spike content of 2,000 mg kg^{-1}, was attributed to loss of soluble Pb during preparation of spiked soils. All test soils were within 10% of the mean Pb content (Table 2). The total Pb concentration of test soils ranged from 1700 to 2020 mg Pb kg^{-1}. The mean $Ca(NO_3)_2$-extractable Pb concentration was 477 mg kg^{-1} and

ranged from 0.6–64% of total Pb levels (Table 2). Conder and Lanno (2000) found that $Ca(NO_3)_2$-extractable Pb levels in artificial soils were 0.4-3.0% of total Pb levels. The 100-fold difference found for $Ca(NO_3)_2$-extractable Pb concentrations expressed as a percent of total Pb in this study suggests that soil properties profoundly modulated extractable Pb levels.

Extraction techniques using weak salt solutions (< 1*M* $CaCl_2$ or $Ca(NO_3)_2$) estimate metal levels in soil pore water and readily dissolved metal adsorbed to soil components or in minerals with high water solubility. These forms of extractable metal are a more accurate measure of actual metal exposure than total metal levels (Lanno et al., 1999). This type of extraction technique has shown promise as a toxicity-related measure of bioavailability in soils (Conder and Lanno, 2000; Basta and Gradwohl, 2000; Posthuma et al., 1997; Sloan et al., 1997; Peijnenburg et al., 1997, 1999b; Weljte, 1998).

Because surrogate measures of bioavailability must be correlated with organismal responses (Lanno et al., 2002, in press), models were developed using both direct (internal concentrations) and indirect approaches (organismal responses and weak electrolyte extractions) for assessing Pb bioavailability and toxicity. Models were developed for earthworm mortality, cocoon production, internal concentrations, extractable Pb, and bioaccumulation factors. Path analysis results for soils tested are listed in Table 3. Simple correlation coefficient (*r*) values between pH, OC, clay, and extractable Pb concentrations

are listed for comparison with path analysis results. Path analysis partitions each *r*-value into one direct effect (underlined, main diagonal positions) and two indirect effects (off diagonal positions). Significant direct effects are indicated by corresponding *p*-values for each model tested. Significant coefficient of determination (R^2) values and low uncorrelated (U) values indicate that the path analysis model explains most of the variation in each of the models tested. The model explains most of the variation observed in $Ca(NO_3)_2$-extractable Pb (R^2 = 0.90, $P < 0.01$) (Table 3). Simple correlation results (r-values) indicate that that soil pH, OC, and clay strongly affected $Ca(NO_3)_2$-extractable Pb ($P < 0.01$). Path analysis direct effects also imply that soil pH, OC, and clay strongly affected $Ca(NO_3)_2$-extractable Pb ($P < 0.01$). According to Adriano (2001), high pH, OM, and clay content reduces the extractability of Pb in soils.

Regression analysis results for the 22 Pb spiked soils tested are listed in Table 4. Backwards-stepwise regression was used to identify the critical soil properties that explain most of the variation of these parameters in 22 field soils. Backwards stepwise regression results indicate that pH, OC, and clay explained the variance among $Ca(NO_3)_2$-extractable Pb concentrations (R^2 = 0.96, $P <$ 0.01) and were highly predictive of available Pb in soil.

Earthworm mortality

Cumulative mean (n = 66) earthworm mortality was 1.2%, which was < 10% in each of the 22 unspiked reference soils. Earthworm mortality ranged from zero

mortality to complete acute mortality when exposed to Pb spiked soils (Figure 2). Adverse physiological responses to Pb exposure included dermal lesions and yellow secretions, typical of stress responses in *E. andrei* (Edwards and Bohlen, 1992). In soils with 100% mortality (Bernow B, Norge A, Teller A, Pratt A, and Pratt B), earthworms died during the first week of the experiment. Soils with low pH and clay content resulted in many dead animals within the first day. This was the case for Norge A and Teller A soils which had 40% and 50% mortality, respectively, by day 2. In Norge and Teller, all worms were dead by day 2 and day 4, respectively. According to Spurgeon et al. (1994), this suggests that the main toxic effect was exerted by uptake across the body wall, rather than via dietary metal assimilation. Similar results were reported for *Eisenia fetida* exposed to artificial soil spiked at a level of 10,000 mg Pb kg^{-1} (Spurgeon et al., 1994). However, less than 10% mortality occurred at 2,000 mg Pb kg^{-1} in the artificial soil tested in that particular study. The range of mortality observed in our study was the result of differences in Pb bioavailability due to Pb interactions with the soil properties, assuming similar behavior of earthworms in each soil.

Estimated LC_{50} value for probability of earthworm mortality based on $Ca(NO_3)_2$-extractable Pb was 437 mg Pb kg^{-1} (Figure 3). Complete mortality (100%) was observed in soils with $Ca(NO_3)_2$-extractable Pb levels > 900 mg kg^{-1}. The 100-fold difference of $Ca(NO_3)_2$-extractable Pb and complete mortality in soils with > 900-mg kg^{-1} $Ca(NO_3)_2$-extractable Pb are evidence of the effects of soil properties modifying environmentally available Pb. Results show a significant

relationship ($P < 0.01$) for $Ca(NO_3)_2$-extractable Pb and mortality (Figure 4). Results support the use of weak-electrolyte extractions as a surrogate measure of Pb bioavailability.

The model explains the variation observed in earthworm mortality ($R^2 = 0.76$, $P < 0.01$) (Table 3). Simple correlation results (*r*-values) indicate that soil pH, OC, and clay significantly affected mortality ($P < 0.01$). However, OC did not retain significance when partitioned into direct and indirect effects by path analysis. Simple correlation coefficients are significant due to indirect effects or intercorrelations of soil properties. Path analysis partitioning provides direct effects or causation of soil properties on mortality. The path analysis partitioning shows strong pH and clay direct effects ($P <0.01$) on mortality.

Backwards stepwise regression results reveal that OC, and interactions of pH-clay and OC-clay, was more important than clay or pH alone for modifying Pb bioavailability for mortality ($R^2 = 0.79$, $P < 0.01$) (Table 4). Clay and pH have been reported in regression models as the most important soil properties modifying Pb uptake rates (Peijnenburg et al. 1999b, Janssen et al., 1997). Soils containing higher clay content have a higher binding capacity for Pb (Adriano, 2001), potentially causing Pb to be unavailable for uptake by organisms. According to Adriano (2001), the affinity for Pb is typically as follows: clay > silt > sand.

Internal concentrations

The mean (SD) internal concentration of *E. andrei* exposed to unspiked reference soils was 0.60 mg Pb kg^{-1} (0.65) (Table 5). The mean Pb concentration of earthworms exposed to 2,000 mg Pb kg^{-1} was 296 mg kg^{-1} (261). A 28-fold difference in concentration in earthworms exposed to the same total soil content of Pb in 22 soils indicates that soil properties are modifying the uptake of Pb. This is consistent with the findings of Peijnenburg et al. (1999b), indicating that soil properties have a significant impact on Pb uptake by *E. andrei.*

Earthworms exposed to soils where 100% mortality occurred were removed from internal Pb concentration statistical models to avoid comparison of depurated and non-depurated worms because there may be differences in the soil content of the earthworm gut and differences in Pb uptake due the physiological effects of acutely toxic Pb exposure. The model explains most of the variation observed in internal concentrations (R^2 = 0.60, $P < 0.01$) (Table 3). Correlation results indicate that pH, OC, and clay significantly influenced internal concentrations ($P < 0.05$). Clay and OC do not remain significant when partitioned into causal or direct effects by path analysis. Path analysis partitioning shows pH is a causative or direct effect ($P < 0.05$) on internal Pb concentrations. Previous research has indicated that pH is typically the main factor modulating metal solubility, bioavailability, and uptake (McLean and Bledsoe, 1992; Peijnenburg et al., 1997, 1999b; Posthuma et al., 1997; Basta et al., 1993; Smit et al., 1998).

Backwards-stepwise regression results indicate that pH and OC contribute most to explaining the variance among worm concentrations (R^2 = 0.72, $P < 0.01$) (Table 4). In a previous study, regression formulae suggest the most significant impact on Pb uptake is pH and clay and that Pb is primarily taken in via the labile (extractable) soil fraction (Peijnenburg et al. 1999b). Regression analysis results established a relationship (r^2 = 0.64) for 0.5 M $Ca(NO_3)_2$-extractable Pb and internal Pb concentrations ($P < 0.01$) (Figure 5).

Average internal concentrations of earthworms exposed to soils with $Ca(NO_3)_2$-extractable Pb > 900 mg kg^{-1} had higher Pb concentrations than worms exposed to soils with lower $Ca(NO_3)_2$-extractable Pb levels. The main detoxification pathways for Pb are thought to be sequestration within inorganic matrices or binding to organic ligands (Spurgeon and Hopkin, 1999). The main site of storage is the chloragogenous tissue that contains granules of phosphate-rich complexes containing calcium and zinc (Morgan and Morgan, 1988). These granules are involved in binding borderline metals such as Pb by exchanging matrix-associated calcium (Morgan and Morgan, 1988, 1993). Adverse effects can be expected only when the capacity of detoxification mechanisms is exceeded, occurring at a very high internal concentration when metal is slowly sequestered (Lock, 2001). Resulting mortalities in many of the soils tested suggests that detoxification mechanisms were exceeded. A significant

relationship was found for internal concentrations and mortality (r^2 = 0.62, P < 0.01).

Cocoon production

Cocoon production of *E. andrei* decreased with increasing internal concentrations (Figure 6). Similar results were observed for *Eisenia fetida* cocoon production when exposed to Cd (Lock et al., 2001). The model explains most of the variation observed in cocoon production (R^2 = 0.67, P < 0.01) (Table 3). Simple correlation results reveal that pH, OC, and clay influenced cocoon production (P < 0.05). Path analysis partitioning indicated a causative or direct effect of OC on cocoon production (P < 0.05). The natural habitats for *E. andrei* are those of very high organic matter such as manure or compost piles (ASTM, 1997). Backwards-stepwise regression indicates OC and clay contribute most to explaining the variance among cocoon production (R^2 = 0.92, P < 0.01) (Table 4).

Biota-soil accumulation factors

Biota-soil accumulation factors (BSAFs) were developed for this study based on total Pb concentrations ($BSAF_{Total}$) and weak electrolyte extractions ($BSAF_{Ca(NO3)2}$). Biota-soil accumulation factors represent the ratio of internal Pb concentrations in exposed earthworms to concentrations in the exposure matrix. Calculated $BSAF_{Total}$ and $BSAF_{Ca(NO3)2}$ values are listed in Table 6. Biota-soil accumulation factors ranged approximately 10-fold from 0.02 – 0.30 for $BSAF_{Total}$ and over two orders of magnitude from 0.06 – 7.14 for $BSAF_{Ca(NO3)2}$. Because

BSAFs are assumed to be independent of soil Pb concentrations, they are often used to assess the effect of soil properties on bioavailability (Peijnenburg et al., 1999a, 1999b; Janssen, 1997). Results show $BSAF_{Ca(NO3)2}$ values decreased according to a power function with increasing extractable Pb concentrations (Figure 7).

The significant coefficient of determination (R^2) and low uncorrelated (U) values indicate that the model explains most of the variation in the $BSAF_{Ca(NO3)2}$ model (Table 3). Simple correlation results indicated a significance of pH on $BSAF_{Ca(NO3)2}$ ($P < 0.01$). Path analysis partitioning for $BSAF_{Ca(NO3)2}$ indicates that pH and clay are significant causative or direct effects on $BSAF_{Ca(NO3)2}$ ($P < 0.01$ and $P < 0.05$, respectively). Backwards-stepwise regression results suggest that clay, in addition to pH and OC, is significant in explaining the variance in $BSAF_{Ca(NO3)2}$ ($R^2 = 0.91$, $P < 0.01$) (Table 4). The variance in bioaccumulation factors for Pb was best explained by soil pH (Peijnenburg et al. 1999b). Previous earthworm bioaccumulation factor models have shown that low pH increases the uptake of Pb while high soil organic matter reduces bioavailability (Corp and Morgan, 1991; Ma, 1982). Regression models developed in this study are consistent with these findings. However, path analysis models generated indicate that pH is significant for bioaccumulation factors based on total Pb and pH and clay are significant for bioaccumulation factors based on $Ca(NO_3)_2$-extractable Pb.

Bioaccumulation factors are frequently used to assess the effect of soil-modifying factors on the bioavailability of metals in soils (Janssen et al., 1997; Peijnenburg et al., 1999a, 1999b). However, there is much debate about the use of bioaccumulation factors to assess the bioavailability of metals. Some authors report that bioaccumulation factors should be questioned because they tend to decrease with increasing metal concentrations, indicating there is no relationship between the internal concentration and the bioavailable concentration (Lock, 2000). Others state that bioaccumulation factors are more appropriate than body concentrations for normalization among field soils (Janssen, 1997). Bioaccumulation factors are usually normalized to total metal concentrations in soil. Peijneburg et al. (1999), suggests bioaccumulation factors should be based on bioavailable concentrations in the soil. In this study, bioaccumulation factors based on $Ca(NO_3)_2$-extractable Pb were found to decrease with increasing concentrations of available Pb. It is assumed that bioaccumulation occurs when BSAFs are greater than one (Lock, 2001). Bioaccumulation factors based on total Pb in this study were consistently less than unity in all soils. However, bioaccumulation factors based on available concentrations in this study found many of the values were greater than one. In soils with the largest $BSAF_{Ca(NO3)2}$ values, little or no mortality was observed suggesting that $BSAF_{Ca(NO3)2}$ are poor indicators of adverse effects of Pb to earthworms ($P = 0.15$).

Empirical formulas developed may be useful for predicting the potential environmental risks of Pb in soil. Path analysis models proved useful for

providing a quantitative causal influence of Pb bioavailability and toxicity to earthworms. pH was the most important soil property modifying the bioavailability and toxicity of Pb. Conclusions of our study support the use of weak-electrolyte extractions as a surrogate measure of bioavailability. The significant relationship found for internal concentrations and mortality suggests that internal concentrations may prove useful as indicators of adverse effects of Pb toxicity and bioavailability to earthworms. Biota-soil accumulation factors in this study were deemed as poor indicators of environmental risk of Pb toxicity. Furthermore, the decrease in $BSAF_{Ca(NO3)2}$ with increasing available Pb concentrations indicates that BSAFs should not be used assess the influence of soil properties on Pb bioavailability.

Acknowledgements

We would like to thank Derrick Chappell for assistance with bioassays. The research in this manuscript has been funded by the U.S. Environmental Protection Agency, Office of Research and Development, National Center for Exposure Assessment. Although the research described in this paper has been funded by the U.S. EPA to Dr. Roman Lanno and Dr. Nicholas Basta, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

Adriano, DC. 2001. *Trace elements in terrestrial environments.* Springer, New York, USA.

American Society for Testing and Materials. 1997. Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with Lumbricid earthworm *Eisenia fetida*, standard E 1676-97. In: Annual Book of ASTM Standards, pp. 1056-1074.

Basta NT, Pantone DJ, Tabatabai MA. 1993. Path analysis of heavy metal adsorption by soil. *Agron. J.* 85:1054-1057.

Basta NT, Gradwohl R. 2000. Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soil by a sequential extraction procedure. *Journal of Soil Contamination.* 9:149-164.

Beyer WN, Hensler G, Moore, J. 1987. Relation of pH and other soil variables to concentrations of Pb, Cu, Zn, Cd, and Se in earthworms. *Pedobiologia.* 30:167-172.

Conder JM, Lanno RP. 2000. Evaluation of cadmium, lead, and zinc to *Eisenia fetida*. *Chemosphere.* 41:1659-1668.

Corp N, Morgan AJ. 1991. Accumulation of heavy metals from polluted soils by the earthworm, Lumbricus rubellus: can laboratory exposure of 'control' worms reduce biomonitoring problems? *Environ. Poll.* 74:39-52.

Edelman WMT, Van Beersum I, Jans T. 1983. Uptake of Cd, Zn, Pb, and Cu by earthworms near a zinc smelting complex: influence of soil pH and organic matter. *Bull. Environ. Contam. Toxicol.* 30:424-427.

Edwards CA, Bohlen PJ. 1992. The effects of toxic chemicals on earthworms. *Rev. Environ. Contam. Toxicol.* 125:23-99.

Gee GW, Bauder JW. 1986. Particle-size analysis. In A. Klute, ed., *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods,* 2nd ed. Agronomy Monograph 9. Soil Science Society of America, Madison, WI, USA, pp. 383-411.

Heanes DL. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Commun. In Soil Sci. Plant Analysis.* 15:1191-1213.

Hendershot WH, Duquette M. 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Sci. Soc. Am. J.* 50:605-608.

Janssen RPT, Posthuma L, Baerselman R, Den Hollander HA, Van Veen RPM, Peijnenburg WJGM. 1997. Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal accumulation in earthworms. *Environ. Toxicol. Chem.* 16:2479-2488.

Lanno R. Ed. 2002 (In press). *Contaminated soils: From soil-chemical interactions to ecosystem management. Proceedings of Workshop on Assessing Contaminated Soils, Pellston, MI, 23-27 September 1998.*

Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, USA.

Lanno RP, Conder JM, Seals L. 1999. Critical limits for heavy metals in soils and surface waters. *Workshop on effects-based approaches for heavy metals*. Federal Environmental Agency (Umweltbundesamt), Berlin, October.

Lanno RP, McCarty LS. 1997. Earthworm bioassays: Adopting techniques from aquatic toxicology. *Soil Biol. Biochem.* 29:693-697.

Lock K, Janssen CR. 2001. Zinc and cadmium body burdens in terrestrial Oligochaetes: use and significance in environmental risk assessment. *Environ. Toxicol. Chem.* 20:2067-2072.

Lock K, Janssen CR, De Coen WM. 2000. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to *Enchytraeus albidus*. *Environ. Toxicol. Chem.* 19:2666-2671.

Loehlin JC. 1987. *Latent variable models: an introduction to factor, path, and structural analysis*. Lawrence, Hillsdale, New Jersey, USA.

Ma WC. 1982. The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. *Pedobiologia* 24:109-119.

McBride MB. 1989. Reactions controlling heavy metal solubility in soils. *Adv. Soil Sci.* 10:1-56.

McLean LE, Bledsoe BE. 1992. Ground water issue: Behavior of metals in soils. EPA/540/S-92/018. U.S. Environmental Protection Agency, Washington, DC.

Morgan ME, Morgan AJ. 1988. Earthworms as biological monitors of Cd, Cu, Pb, and Zn in metalliferous soils. *Environ. Pollut.* 54:123-138.

Morgan AJ, Morris B. 1982. The accumulation and intracellular compartmentation of cadmium, lead, zinc, and calcium in two earthworm species (*Dendrobaena rubida* and *Lumbricus rubellus*) living in highly contaminated soil. *Histochem.* 75:269-285.

Peijnenburg WJGM, Posthuma L, Zweers PGPC, Baerselman R, de Groot AC, van Veen RPM, Jager T. 1999a. Prediction of metal bioavailability in Dutch field soils for the Oligochaeta *Enchytraeus crypticus*. *Ecotoxicol. Environ. Safety* 43:170-186.

Peijnenburg WJGM, Baerselman R, de Groot AC, Jager T, Posthuma L, Van Veen RPM. 1999b. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. *Ecotoxicol. Environ. Saf.* 44:294-310.

Peijnenburg WJGM, Posthuma L, Eijsackers HJP, Allen HE. 1997. A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicol. Environ. Safety* 37:163-172.

Polemio M, Rhoades JD. 1977. Determining cation exchange capacity: a new procedure for calcareous and gypsiferous soils. *Soil Sci. Soc. Am. J.* 41:524-528.

Posthuma L, Baerselman R, Van Veen RPM, Dirven-Van Breemen EM. 1997. Single and joint toxic effects of copper and zinc on reproduction of

Enchytraeus crypticus in relation to sorption of metals in soils. *Ecotoxicol. Environ. Safety* 38:108-121.

Rieuwerts JS, Thornton I, Farago ME, Ashmore, MR. 1998. Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chem. Spec. Bioavail.* 10:61-75.

Schroder J. 2002 (In preparation). Ph.D. Dissertation. Oklahoma State University, Stillwater, OK.

Sloan JJ, Dowdy RH, Dolan MS, Linden DR. 1997. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. *J. Environ. Qual.* 26:966-974.

Smit CE, Posthuma L, Folkerts AJ, Baerselman R, Dirven-Van Breemen EM, Bogte J, Van Beelen P. 1998. Heavy metal behavior in OECD artificial soil, Budel gradient soils and experimental field plot soil-methodological aspects and soil chemical characterization of contamination. In: Posthuma L, Van Gestel CAM, Smit CE, Bakker DJ, Vonk JW. eds, *Validation of Toxicity Data and Risk Limits for Soils: Final Report.* National Institute of Public Health and Environment (RIVM), Bilthoven, The Netherlands, p. 230.

Spurgeon DJ, Hopkin SP. 1999. Tolerance to zinc in populations of the earthworm *Lumbricus rubellus* from uncontaminated and metal-contaminated ecosystems. *Arch. Environ. Contam. Toxicol.* 37:332-337.

Spurgeon DJ, Hopkin SP. 1996. Effects of variations of the organic matter content and pH of soils on the availability and toxicity of zinc to the earthworm *Eisenia fetida*. *Pedobiologia* 40:80-96.

Spurgeon DJ, Hopkin SP, Jones DT. 1994. Effects of Cd, Cu, Pb, and Zn on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ. Poll.* 84:123-130.

Thomas GW. 1996. Soil pH and soil acidity. In Sparks DL, ed, *Methods of Soil Analysis.* Part 3 Chemical Methods-SSSA Book series no. 5. Soil Science Society of America, Madison, WI, pp. 475-490.

U.S. Environmental Protection Agency. 1994. Method 3051: Microwave assisted acid digestion of sediment, sludges, soils, and oils.

U.S. Environmental Protection Agency. 2000. Ecological soil screening level guidance. Draft Report. Office of Emergency and Remedial Response. Washington, D.C.

Van Gestel CAM, Dirven-van Breeman EM, Baerselman R. 1993. Accumulation and elimination of cadmium, chromium, and zinc and the effects on growth and reproduction of *Eisenia andrei* (Oligochaeta, Annelida). *Sci. Total Environ. Supplement* 585-597.

Weljte L. 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb, and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. *Chemosphere* 36:2643-2660.

Williams WA, Jones MB, Demment MW. 1990. A concise table for path analysis statistics. *Agron. J.* 82:1022-1024.

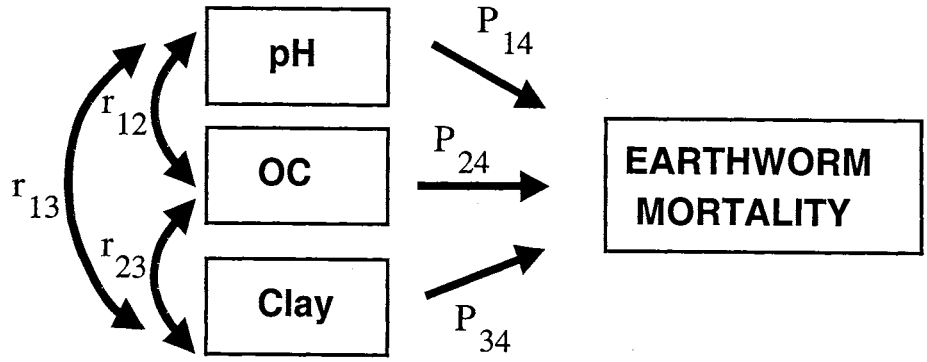

Figure 1. Path diagram for the effect of soil properties on earthworm mortality.

Table 1. Properties of Soils

Soil	Horizon	Unspiked Soil pH[a]	Pb spiked soil pH[a]	CEC cmol/kg[b]	OC	Sand	Clay	Silt	Class
						%			
Bernow	B	4.9	4.1	6.74	0.30	58.8	26.3	17.5	Sandy Clay Loam
Canisteo	A	7.5	7.6	30.5	3.00	31.3	38.8	51.3	Silt Loam
Dennis	A	5.6	4.8	9.77	1.90	37.5	23.8	40.0	Loam
Dennis	B	6.1	5.2	14.6	0.80	21.3	45.0	40.6	Clay Loam
Doughtery	A	5.3	4.5	3.33	1.20	75.0	11.3	21.3	Loan Sand
Hanlon	A	7.4	6.7	16.3	1.60	63.8	17.5	23.8	Sandy Loam
Kirkland	A	5.6	4.8	14.0	1.45	12.5	31.3	57.5	Silty Clay Loam
Luton	A	7.1	6.6	32.4	2.00	2.50	71.3	38.8	Clay
Mansic	A	7.8	7.7	16.5	1.50	33.8	30.0	43.8	Loam
Mansic	B	8.0	7.8	11.7	0.53	30.0	35.0	42.5	Clay Loam
Norge	A	4.0	3.8	4.57	1.20	36.9	17.5	45.6	Silt Loam
Osage	A	6.6	5.9	28.3	2.60	13.8	55.7	53.8	Silty Clay Loam
Osage	B	6.8	5.9	27.5	2.00	11.3	61.3	47.5	Silty Clay
Pond Creek	A	5.2	4.1	10.7	1.90	16.3	28.8	62.5	Silt Loam
Pond Creek	B	6.0	5.2	12.5	0.80	18.8	32.5	48.8	Silty Clay Loam
Pratt	A	6.5	4.6	4.40	0.90	90.0	5.00	3.80	Silt
Pratt	B	6.4	5.2	3.40	0.50	92.5	6.25	1.30	Silt
Richfield	B	7.7	6.4	22.3	1.10	11.3	41.3	51.3	Silty Clay Loam
Summit	A	7.2	7.0	29.4	2.40	17.5	45.7	53.8	Silty Clay Loam
Summit	B	7.1	6.5	27.6	1.25	10.0	56.8	48.8	Silty Clay
Taloka	A	5.1	4.2	4.85	1.20	20.0	11.3	58.8	Silt Loam
Teller	A	4.5	4.3	3.01	0.85	66.9	10.0	23.8	Silt Loam
MINIMUM		4.0	3.8	3.01	0.30	2.50	5.00	1.30	
MAXIMUM		8.0	7.8	32.4	3.00	92.5	71.3	62.5	
MEAN		6.3	5.6	15.2	1.41	35.1	32.0	40.0	
MEDIAN		6.5	5.2	13.3	1.25	25.7	30.7	45.0	

[a] pH determined by 1:1 soil:water
[b] Cation Exchange Capacity measured using 0.1 M $BaCl_2$ for non-calcareous soils; 1 M NaOAc, pH 8.2 for calcareous soils.

Table 2. Pb soil concentrations

Soil	Horizon	$Ca(NO_3)_2$-extractable Pb[1] mg kg^{-1}	%RSD	%[2]	Total Pb[3] mg kg^{-1}	%RSD
Bernow	B	1,220	0.41	64.0	1,910	4.74
Canisteo	A	10.7	0.55	0.59	1,820	5.25
Dennis	A	598	2.78	31.1	1,920	1.27
Dennis	B	596	5.45	30.6	1,950	0.82
Dougherty	A	800	8.45	43.4	1,840	6.40
Hanlon	A	70.3	1.33	3.88	1,810	1.01
Kirkland	A	665	2.43	35.4	1,880	0.25
Luton	A	17.6	8.52	0.98	1,800	0.07
Mansic	A	26.1	8.46	1.29	2,020	0.32
Mansic	B	40.5	12.5	2.39	1,700	3.70
Norge	A	990	4.70	53.7	1,840	6.85
Osage	A	69.1	3.00	3.80	1,820	3.66
Osage	B	74.4	5.37	4.11	1,810	0.43
Pond Creek	A	511	9.42	26.9	1,900	3.87
Pond Creek	B	705	1.15	37.2	1,900	0.87
Pratt	A	1,090	1.22	54.1	2,010	1.45
Pratt	B	1,050	3.23	56.7	1,850	4.66
Richfield	B	151	0.57	8.14	1,860	0.48
Summit	A	21.7	7.59	1.12	1,940	0.73
Summit	B	78.3	16.8	4.07	1,930	0.15
Taloka	A	726	9.50	36.6	1,980	0.54
Teller	A	977	2.79	53.9	1,810	0.12
MINIMUM		10.7	0.41	0.59	1,700	0.07
MAXIMUM		1,220	16.8	64.0	2,020	6.85
MEAN		477	5.29	25.2	1,880	2.17
MEDIAN		554	3.97	28.8	1,870	0.94

[1] Extracted using 0.5 M $Ca(NO_3)_2$, mean (n=3)
[2] Percent of total metal that was $Ca(NO_3)_2$-extractable
[3] Extracted according to EPA Method 3051 and measured by ICP-AES, mean (n=2)

Table 3. Path analysis direct effects (diagonal, underlined) and indirect effects (off diagonal) of soil pH, organic carbon (% OC), and clay (mmol kg^{-1}) on extractable Pb concentrations and *Eisenia andrei* after 28-day exposure to Pb.

Response		pH	OC	Clay	r	R^2	U
$Ca(NO_3)_2$	pH	-0.62**	-0.10	-0.14	-0.86**	0.90**	0.32
extractable Pb	OC	-0.23	-0.27**	-0.13	-0.63**		
(mg/kg)	Clay	-0.33	-0.13	-0.27**	-0.72**		
% Mortality	pH	-0.40**	-0.06	-0.26	-0.72**	0.76**	0.49
	OC	-0.15	-0.16	-0.24	-0.55**		
	Clay	-0.21	-0.08	-0.50**	-0.78**		
Internal	pH	-0.43*	-0.09	-0.10	-0.62**	0.60**	0.63
concentration[a]	OC	-0.12	-0.34	-0.10	-0.55*		
(mg/kg dry wt.)	Clay	-0.14	-0.11	-0.29	-0.54*		
Cocoon	pH	0.26	0.25	0.02	0.52*	0.67**	0.58
Production[a]	OC	0.10	0.66*	0.02	0.78**		
	Clay	0.13	0.32	0.04	0.50*		
$BSAF_{Ca(NO3)2}$[b]	pH	0.84**	0.07	-0.12	0.79**	0.75**	0.50
	OC	0.24	0.25	-0.12	0.37		
	Clay	0.28	0.08	-0.36*	0.00		

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Cumulative mean (3 replicates per soil-Pb combination)

[b] Soils with 100% mortality removed from model

Table 4. Multiple regression formulae describing the quantitative relationship between soil properties, *Eisenia andrei* after 28-day exposure to Pb, and extractable Pb concentrations.

Response	Regression equation obtained[a]	Statistics
$Ca(NO_3)_2$-extractable (mg Pb kg^{-1})	$y = 2938 - 330.9(pH) - 948.6(OC) - 5.092(Clay) + 87.77(OC)^2 + 81.82(pH*OC)$	$R^2 = 0.96$, n = 22, $P < 0.0001$
% Mortality	$y = 133.0 - 44.21(OC) - 0.428(pH*Clay) + 0.975(OC*Clay)$	$R^2 = 0.79$, n = 22, $P < 0.0001$
Internal concentration[b] (mg Pb kg dry $wt.^{-1}$)	$y = 3430 - 975.9(pH) - 93.13(OC) + 74.37(pH)^2$	$R^2 = 0.72$, n = 17, $P = 0.0007$
Cocoon Production[b]	$y = 3.92 - 8.47(OC) - 0.21(Clay) + 4.77(OC)^2 + 0.01(Clay)^2 + 0.57(pH*OC) - 0.14(OC*Clay)$	$R^2 = 0.92$, n = 17, $P < 0.0001$
$BSAF_{Ca(NO3)2}$[b]	$y = 13.91 - 5.753(pH) + 0.517(pH)^2 - 0.002(Clay)^2 + 0.143(pH*OC) + 0.022(pH*Clay)$	$R^2 = 0.91$, n = 17, $P < 0.0001$

[a] All variables in the models are significant ($P < 0.1$)

[b] Soils with 100% mortality were removed from model

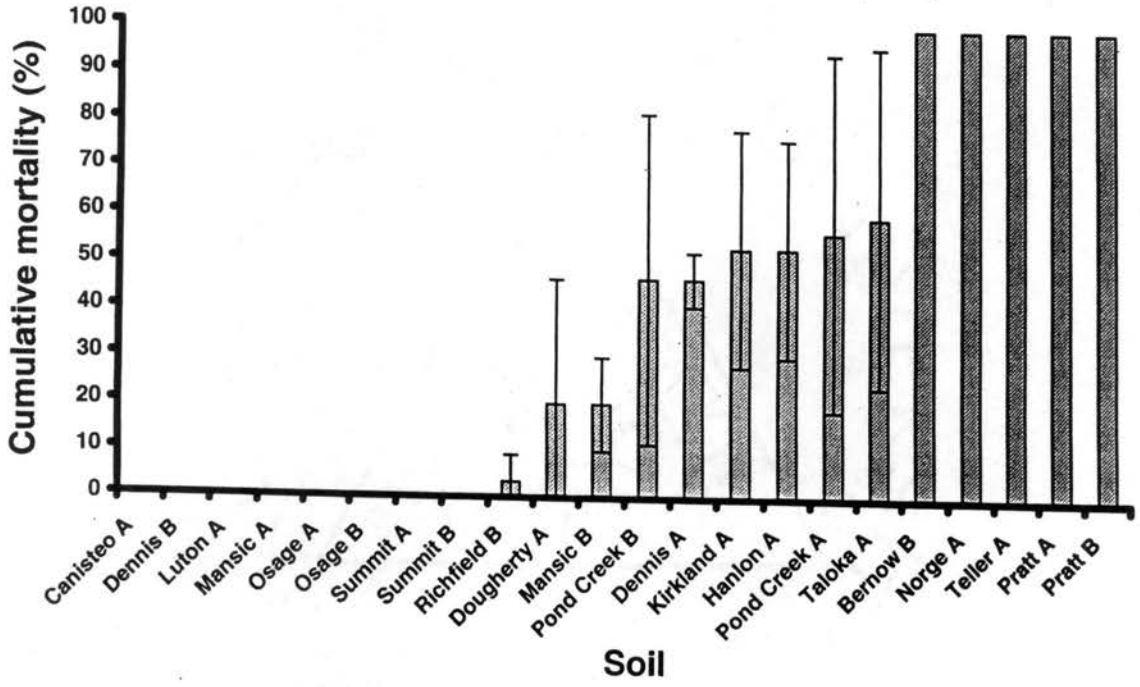

Figure 2. Cumulative mortality (mean of three replicates, ± 95% CI) of *Eisenia andrei* exposed to 2,000 mg Pb/kg spiked soils for 28 days.

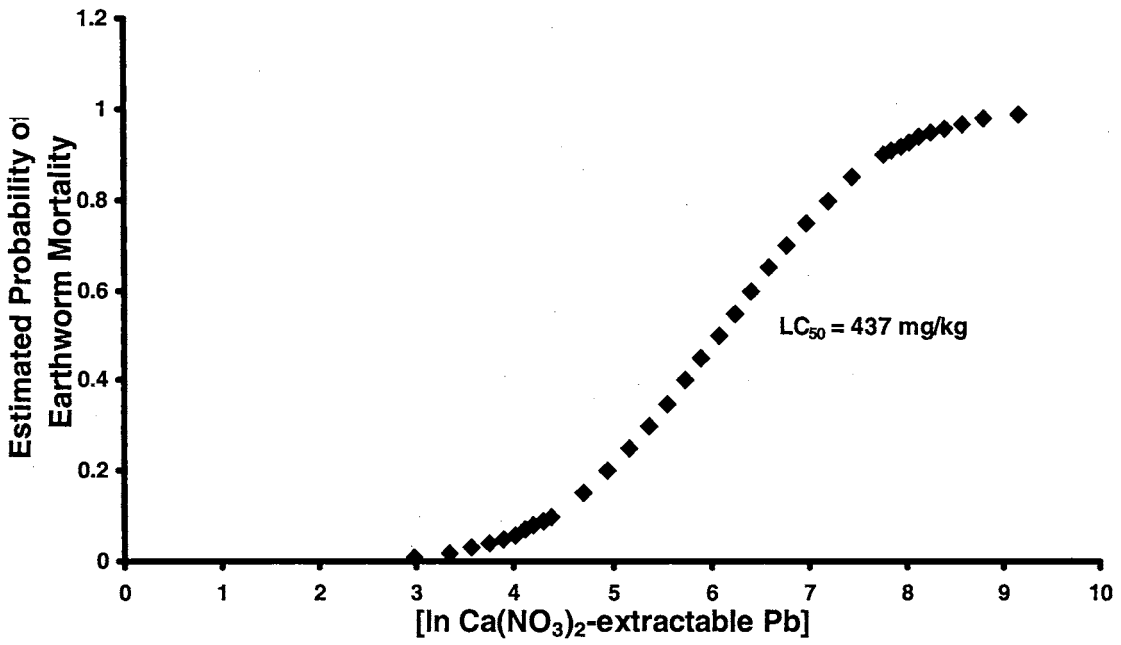

Figure 3. Estimated probability of earthworm mortality based on natural log $Ca(NO_3)_2$-extractable Pb ($\chi^2 < 0.0001$).

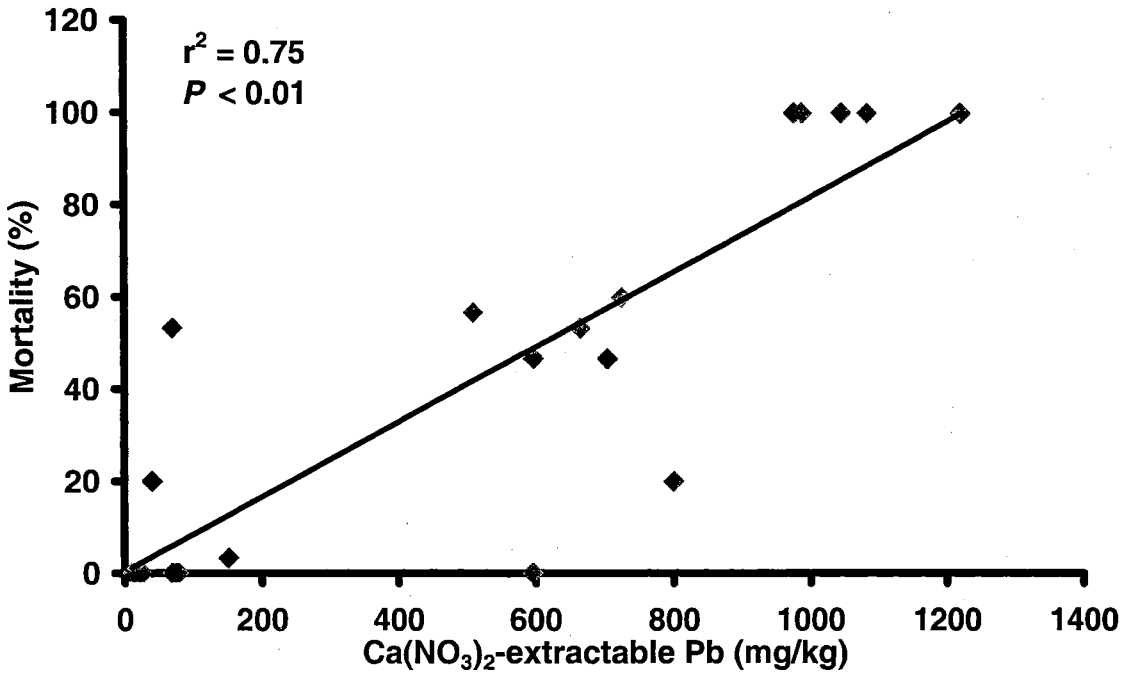

Figure 4. Cumulative (◆ mean of 3 replicates per Pb-soil combination) mortality of *Eisenia andrei* exposed to 22 Pb spiked soils as a function of $Ca(NO_3)_2$-extractable Pb concentrations.

Table 5. Internal concentrations of Eisenia andrei exposed to unspiked and Pb spiked soils (mean, n = 3).

Soil	Horizon	Unspiked soil earthworm Concentration[a] mg kg^{-1}	%RSD	Pb spiked soil earthworm concentration[b] mg kg^{-1}	%RSD
Bernow	B	0.22	48.9	823	20.5
Canisteo	A	0.27	50.6	76.4	3.56
Dennis	A	0.36	51.2	176	63.6
Dennis	B	0.19	44.5	172	26.1
Dougherty	A	0.30	45.0	416	55.0
Hanlon	A	0.28	72.2	183	36.7
Kirkland	A	0.19	76.5	40.6	39.9
Luton	A	0.12	66.4	28.7	25.9
Mansic	A	0.23	93.6	106	47.7
Mansic	B	0.38	51.8	195	60.9
Norge	A	1.90	17.9	215	88.7
Osage	A	0.16	97.6	38.8	53.3
Osage	B	0.24	36.7	38.3	28.1
Pond Creek	A	0.10	59.2	566	41.7
Pond Creek	B	0.11	47.9	423	31.9
Pratt	A	0.50	58.7	782	9.29
Pratt	B	0.38	29.2	701	18.1
Richfield	B	0.08	67.9	261	56.1
Summit	A	0.51	58.0	126	17.8
Summit	B	0.12	64.7	83.1	28.0
Taloka	A	0.59	79.9	366	43.3
Teller	A	5.98	53.7	690	1.47
MINIMUM		0.08	17.9	28.7	1.47
MAXIMUM		5.98	97.6	823	88.7
MEAN		0.60	57.8	296	36.3
MEDIAN		0.26	55.9	189	34.3

[a] Pb concentration in digests of worms exposed to reference (unspiked) soils, measured by HGAAS (limit of detection 0.4 µg/g)

[b] Pb concentration in digests of worms exposed to 2000 mg/kg Pb spiked soils, measured by HGAAS (limit of detection 0.4 µg/g)

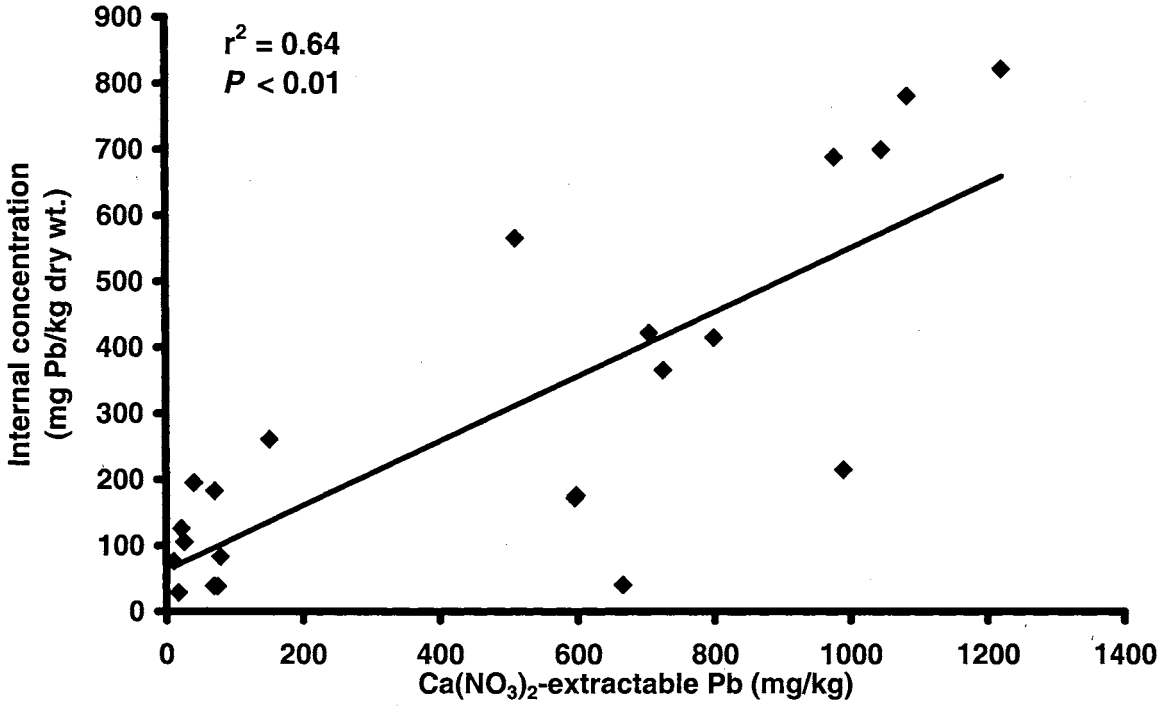

Figure 5. Pb concentrations (◆ mean of 3 replicates per Pb-soil combination) of *Eisenia andrei* exposed to Pb spiked soils versus $Ca(NO_3)_2$-extractable soil Pb concentrations.

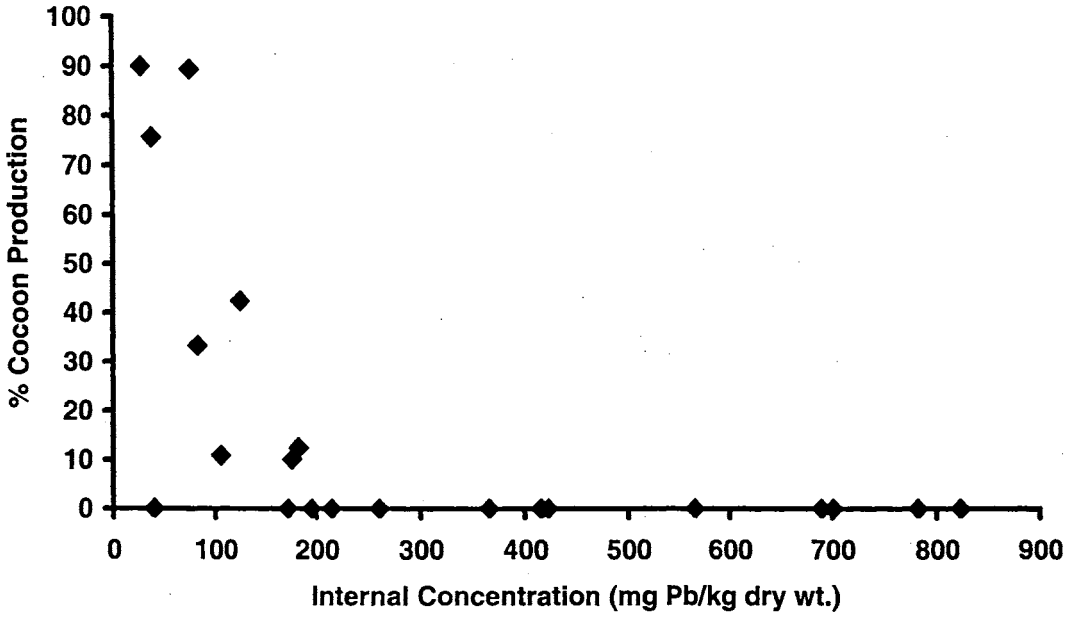

Figure 6. Cocoon production after 28-day exposure, expressed in percentage of control, versus earthworm concentrations.

Table 6. Biota-Soil Accumulation Factors for *Eisenia andrei* after 28-Day exposure to in 22 field soils spiked with Pb.

Soil	Horizon	$BSAF_{Total}$	$BSAF_{Ca(NO3)2}$
		kg_s kg dry $weight_w^{-1}$	kg_s kg dry $weight_w^{-1}$
Bernow	B	0.43	0.67
Canisteo	A	0.04	7.14
Dennis	A	0.09	0.29
Dennis	B	0.09	0.29
Dougherty	A	0.23	0.52
Hanlon	A	0.10	2.60
Kirkland	A	0.02	0.06
Luton	A	0.02	1.63
Mansic	A	0.05	4.04
Mansic	B	0.11	4.82
Norge	A	0.12	0.22
Osage	A	0.02	0.56
Osage	B	0.02	0.51
Pond Creek	A	0.30	1.11
Pond Creek	B	0.22	0.60
Pratt	A	0.39	0.72
Pratt	B	0.38	0.67
Richfield	B	0.14	1.73
Summit	A	0.06	5.80
Summit	B	0.04	1.06
Taloka	A	0.18	0.50
Teller	A	0.38	0.71
MINIMUM		0.02	0.06
MAXIMUM		0.43	7.14
MEAN		0.16	1.65
MEDIAN		0.11	0.69

[a] $BSAF_{Total}$ calculated as mg Pb kg dry weight $worm^{-1}$:Total mg Pb kg dry weight $soil^{-1}$.
[b] $BSAF_{Ca(NO3)2}$ calculated as mg Pb kg dry weight $worm^{-1}$:$Ca(NO_3)_2$-extractable mg Pb kg dry weight $soil^{-1}$.

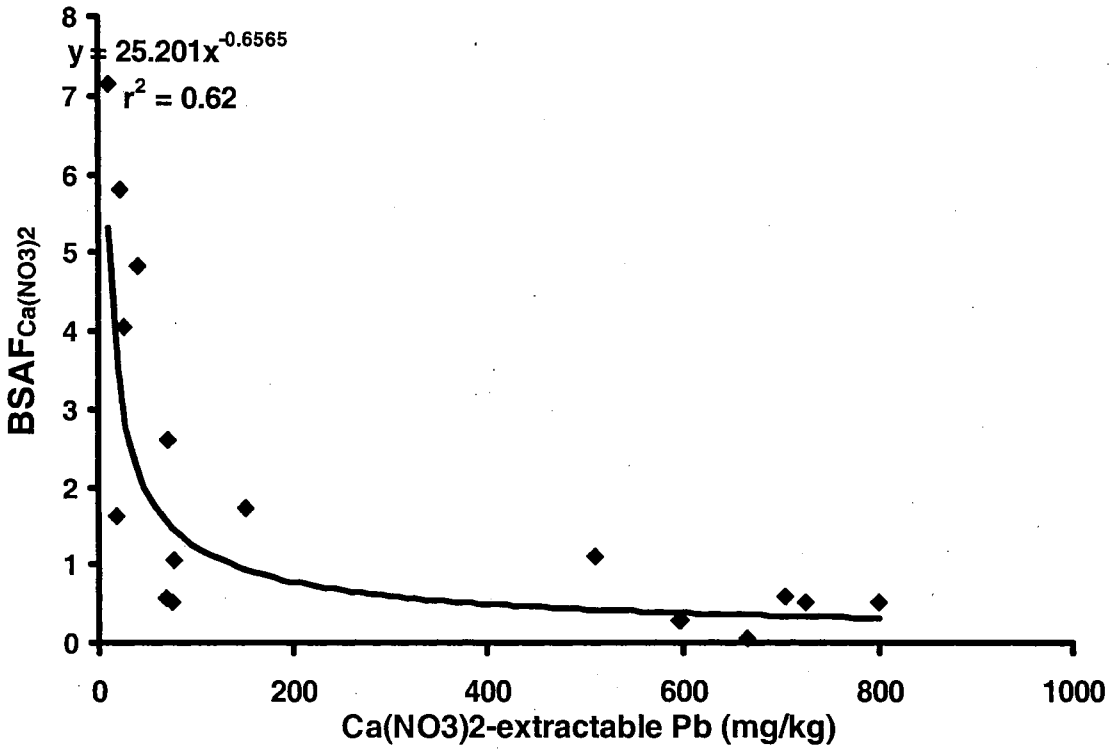

Figure 7. Biota-soil accumulation factors as a function of the $Ca(NO_3)_2$-extractable Pb concentrations in spiked soils.

CHAPTER 2

EFFECT OF SOIL PROPERTIES ON THE BIOAVAILABILITY AND TOXICITY OF CADMIUM TO *EISENIA ANDREI*

ABSTRACT

Cd bioavailability and toxicity is directly influenced by soil properties. In the present study, the relationship between soil properties, and Cd bioavailability and toxicity in earthworms (*Eisenia andrei*) exposed to 22 field soils spiked with Cd is investigated to develop a mathematical model to describe this relationship. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to the same total Cd concentration in spiked soils. Statistical models were developed for earthworm mortality, cocoon production, internal concentrations, extractable Cd levels, and bioaccumulation factors. Soil pH was found to be the most important soil property modulating Cd bioavailability for mortality, internal concentrations, bioaccumulation factors, and extractable Cd levels ($P < 0.05$). Regression analysis results established a relationship ($r^2 = 0.55$) for 0.5 M $Ca(NO_3)_2$-extractable Cd and internal Cd concentrations ($P < 0.01$). Bioaccumulation factors based on total and available Cd in this study were consistently greater than unity in all soils. In soils with the largest bioaccumulation factors, little or no mortality was observed suggesting that bioaccumulation factors are poor indicators of Cd toxicity to earthworms.

INTRODUCTION

Cadmium (Cd) contamination of soil is a worldwide problem that may pose a risk to soil organisms. Sources of Cd contamination of soil include mining and smelter operations, industrial discharge, phosphate fertilizers, nickel-cadmium batteries, and sewage sludge (Adriano, 2001). In order to protect and/or restore soil ecosystems, it is necessary to accurately characterize the risk posed to soil organisms by Cd and other contaminants. Risk is directly related to Cd bioavailability, which depends upon Cd concentration, the geochemical forms of Cd, the species of organism exposed, physical and chemical characteristics of the exposure environment, and the exposure duration. Bioavailability and toxicity are not permanent properties of soil but vary with the interaction between the soil and the organism (Lanno and McCarty, 1997). There are direct and indirect methods for determining bioavailability. Direct measures of bioavailability incorporate organismal responses and/or internal chemical concentrations to estimate bioavailability. Indirect measurements of bioavailability do not use living organisms to estimate the bioavailability of chemicals from soil. Instead, they utilize measured concentrations of chemical species that are potentially available for uptake. Weakly bound or available metals are believed to be available for uptake by earthworms (Posthuma et al., 1997; Peijnenburg et al., 1999b). The authors suggested field soils should be tested to further validate the use of weak electrolyte extractions as surrogate measures of bioavailability. Only an organism can determine bioavailability or toxicity (Lanno and McCarty, 1997).

However, it is necessary to integrate chemical (indirect) and biological (direct) measures to accurately reflect the bioavailability of Cd in soils and to protect ecological receptors.

Soil protection guidelines based on total Cd levels have been suggested for the protection of ecological receptors (USEPA, 2000). Due to soil modifying factors, total Cd concentrations are inaccurate for predicting soil organism toxicity (Ma, 1982; Beyer at al., 1987; Morgan and Morgan, 1988; McLean and Bledsoe, 1992). Risk to soil organisms based on total contaminant concentration is not an accurate predictor of adverse effects (Peijnenburg et al., 1999a) because exposure expressed as total Cd does not consider the effects of soil modifying factors on Cd bioavailability. As an example, soil pH is inversely related to Cd solubility and availability (McBride, 1989; Rieuwerts et al., 1998). However, it is difficult to determine the influence of pH on metal solubility and availability independent from other physicochemical characteristics because so many processes are interrelated (Allen, 2001). Earthworms accumulate Cd more readily in soils with decreased pH and organic matter than in soils with increased pH and organic matter (Edelman et al., 1983). Due to modifying factors, soil metal is often less than 100% available for uptake by organisms (Conder and Lanno, 2000). The objective of this study was to examine the effect of soil properties (e.g., pH, organic matter content, clay content), on Cd bioavailability and toxicity in earthworms (*Eisenia andrei*) and develop a mathematical model to describe this relationship.

METHODS AND MATERIALS

Soil collection and preparation were conducted using standard soil testing methods (see Schroder et al., in preparation, for complete methods). Twenty-two soils with diverse paleoclimatology and geology were collected from Oklahoma and central Iowa to obtain Mollisols with a high organic C content. Soils were spiked with reagent grade $Cd(NO_3)_2$ to obtain soil concentrations of approximately 50 mg Cd kg^{-1} and 300 mg Cd kg^{-1}. Spiked soils were subjected to four wet-dry cycles (see Schroder et al., in preparation, for more detail) to achieve adequate reaction with the soil matrix and reduce the "salt effect" where heavy metal availability is greater in spiked soil than aged contaminated soil with similar metal contamination (Logan and Chaney, 1983). Total Cd in collected soils was determined by an acid digestion microwave technique according to U.S. EPA Method 3051 for confirmation of background Cd concentrations prior to analysis of chemical and physical properties (U.S. EPA 1994). Soil pH was determined in 1:1 soil:water suspension (Thomas, 1996). Soil organic C content was determined by acid dichromate digestion according to Heanes (1984). Cation exchange capacity of non-calcareous soil (soil pH < 7.0) was determined using a procedure adopted from Hendershot and Duquette (1986). Cation exchange capacity of calcareous soils (soil pH > 7.0) was determined according to the method of Polemio and Rhoades (1977). Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). $Ca(NO_3)_2$-extractable Cd was determined by placing soil (1.0 g) in a 50 mL centrifuge tube, extracting with 20.0

mL of 0.5 *M* $Ca(NO_3)_2$ solution, and shaking tubes on a reciprocal shaker for 16 h. The solutions were then centrifuged at 10,000 rpm for 15 min, filtered through a 0.45 μm membrane filter, acidified with 1.0 mL of trace metal concentrated HCl, and stored at 4 °C until analysis of metal by ICP-AES. Spiked soils were digested by microwave according to U.S. EPA Method 3051 to determine total Cd concentrations. Blanks, spikes and certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were digested and analyzed for quality assurance and quality control in the determination of metal content in soil.

Earthworm bioassays

Twenty-eight day bioassays using *Eisenia andrei* were conducted with field soils individually spiked with 50 mg Cd kg^{-1} and 300 mg Cd kg^{-1}. The bioassays were performed in triplicate for each soil-Cd combination and conducted using mature (clitellate) manure worms (*E. andrei*) according to a standard protocol (American Society for Testing and Materials, 1997). The 200-g soil samples were moistened and maintained between 1/3 bar and saturation, placed in glass jars with 3 small air holes in the lid, and acclimated in an environmental chamber maintained at 20±1°C for 24 h prior to the addition of 10 earthworms per replicate. Twenty-four hours prior to the addition of earthworms to test soils, mature (clitellate) earthworms weighing approximately 0.2-0.4 g were removed from synchronized in-house cultures, rinsed with reagent grade water, and placed on moist filter paper for 24 hours to depurate most of the bedding material from their intestinal tracts (Van Gestel et al. 1993). At the start of the toxicity test,

randomly chosen earthworms were removed from the filter paper, rinsed, and separated into replicates of 10 earthworms. Each replicate was blotted dry, weighed, and transferred to one of three jars prepared for each soil. Testing was conducted in an environmental chamber maintained at 20 ± 1°C with constant light. Earthworms were monitored after six hours for physical condition and to determine if burrowing had occurred. Earthworms were observed daily for the first eight days and three times a week thereafter for the remainder of the test to assess the general condition of the worms and remove mortalities. Cocoons were collected on a daily basis by hand sorting. Simultaneously, observations on earthworm performance in ASTM artificial soil (American Society for Testing and Materials, 1997) and unspiked reference soils from each site served as controls for quality assurance with respect to survival, cocoon production, and growth. Artificial soils consisted of 69.5% silica sand, 20% kaolin clay, 10% 2-mm sieved *Sphagnum* peat moss, and approximately 0.5% $CaCO_3$ added to adjust the pH to 7.0±0.5. Reagent grade water was added to hydrate the artificial soil to 45% of its dry weight. All soil materials used were hydrated and allowed to acclimate in the environmental chamber maintained at 20±1°C 24 hours prior to the start of the tests. Earthworms were judged dead if no response was observed after gentle stimulation with a blunt probe. Dead earthworms were removed, rinsed thoroughly with reagent grade water, individually wrapped in aluminum foil, and frozen at -20°C for subsequent analysis. At day 28 of each study, live earthworms were depurated for 24 hours on moist filter paper, rinsed, weighed,

and stored as described above. Upon the completion of toxicity tests, individual soil replicates from all experiments were stored at -20°C in Ziploc® freezer bags.

Internal concentrations

Earthworm Cd concentrations were determined as described by Morgan et al. (1982). An individual worm from each replicate (3 replicates per soil-Cd combination) was removed from the freezer, dried for 24 hours at 105°C in a pre-weighed 10 mL glass beaker, and weighed. Individual worms were then wet digested using 5 mL concentrated trace metal grade HNO_3 (Fisher Scientific). Digests were evaporated to dryness, resolubilized in 3 mL 0.5 M HNO_3, heated for 15 minutes at 60°C, and diluted to final volume of 10 mL with 0.5 M HNO_3. Worm digests were stored in Nalgene® low-density polyethylene bottles until analysis. Cd concentrations in digests were measured using flame AAS (PerkinElmer Analyst 700). The limit of detection for Cd in earthworm tissue digests was 10 μg/L. Cd concentrations in worm tissues were expressed on an mg kg^{-1} dry weight basis. All analyses included procedural blanks, spikes, and certified reference material (lobster hepatopancreas, TORT-2, National Research Council, Canada). Mean (%RSD) spike and certified reference material recoveries were 97 (2.1%) and 96 (1.1%), respectively.

Data analysis

Statistical analyses were performed using PC SAS Version 8.2 (SAS Institute Inc., Cary, NC). LC_{50} values were based on models produced by Proc Probit.

Empirical models were developed for comparison to models in the literature. Backwards-stepwise regression analysis was used to derive empirical models capable of predicting effects of Cd on earthworm mortality, internal concentrations, and cocoon production based on soil properties. The backwards-stepwise regression analysis was used to identify critical soil properties explaining most of the variation. Soil properties that did not explain a significant part of the variation ($P > 0.10$) were not used as independent variables in the multiple regression equation. Statistical models capable of predicting effects of Cd bioavailability, based on soil properties were obtained from the regression analysis. The multiple regression functions have the format:

$$Y = Y = b_0 + b_1(pH) + b_2(clay) + b_3(OC)$$

Where Y = extractable Cd, earthworm response (mortality, internal concentrations, and cocoon production), or biota-soil accumulation factors, A, B, and C = soil properties (pH, clay, OC), and a, b, and c = coefficients.

Empirical models are compared with quantitative causal values for each soil property provided by path analysis models. Path analysis, an extension of the regression model, is a statistical technique that differentiates between correlation and causation (Basta et al., 1993). Path analysis was used to decompose correlations in the model into direct or causal effects of soil properties (Loehlin, 1987) on earthworm mortality, internal concentrations, and cocoon production. Path analysis allows the partitioning of simple correlation coefficients between dependent (e.g. mortality) and independent variables (soil properties) into direct

and indirect effects (Basta et al., 1993). Path analysis also provides a numerical value for each direct and indirect effect and indicates the relative strength of that correlation or causal influence (Loehlin, 1987). Direct effects are standardized partial regression coefficients designated as path coefficients (Basta et al., 1993). Direct and indirect effects are derived from multiple linear regression of soil properties on earthworm response (mortality, internal concentrations, and cocoon production) and simple correlation values between soil properties. In addition, an uncorrelated residual (U) was determined from this model using the following equation:

$$U = \sqrt{1 - R^2}$$

A path analysis model was composed to study the effect of pH, OC, and clay on earthworm mortality (Figure 1). Direct effects (path coefficients) of soil properties on earthworm mortality are represented by the single-headed arrows while the double-headed arrows represent intercorrelation coefficients. Indirect effects of soil properties on earthworm mortality are determined from the product of one double-headed arrow and one single-headed arrow (Basta et al., 1993). Path analysis results were derived using the following equations (Williams et al., 1990):

$$r_{14} = P_{14} + r_{12}P_{24} + r_{13}P_{34} \qquad [1]$$

$$r_{24} = r_{12}P_{14} + P_{24} + r_{23}P_{34} \qquad [2]$$

$$r_{34} = r_{13}P_{14} + r_{23}P_{24} + P_{34} \qquad [3]$$

where r_{i4} corresponds to the simple correlation coefficient between the soil property and earthworm response, P_{i4} are path coefficients (direct effects) of soil

property i on earthworm response, and $r_{ij}P_{i4}$ are the indirect effects of soil property j through i on earthworm response. Subscript designations are: (1) pH, (2) OC, (3) Clay, and (4) earthworm response.

The path analysis results can be presented in a concise table (Williams et al., 1990). This table provides underlined diagonal numbers indicating direct effects and off-diagonal numbers indicating indirect effects. The position of each response in the table corresponds to its position in the matrix of respective equations (equations [1], [2], [3], above). This format allows all potential tables to be presented as one table.

RESULTS AND DISCUSSION

Metal availability

The 22 soils collected had a wide range of soil properties including soil pH (4.0–8.0), cation exchange capacity (3.0 to 32.4 $cmol_c\ kg^{-1}$), organic C (0.3 to 3.0%), and clay content (5.0 to 71%) (Table 7). The Cd content of collected soils was similar to uncontaminated background soil contents prior to Cd amendments (See Schroder et al., in preparation). The target values for Cd amended in the test soils were 50 and 300 mg kg^{-1}, based upon earthworm responses in range-finder tests. The mean total Cd contents in test soils of 47.4 (Table 8) and 302 mg Cd kg^{-1} (Table 9), slightly lower than the target spike content of 50 and 300 mg kg^{-1}, respectively, were attributed to loss of soluble Cd during preparation of spiked soils. All test soils were within 10% of the mean Cd contents. The 50 and

300 mg kg^{-1} total Cd concentrations of test soils ranged from 40.2 to 53.9 mg kg^{-1} and 270 to 326 mg kg^{-1}, respectively. The 50 mg kg^{-1} mean $Ca(NO_3)_2$-extractable Cd concentration was 27.2 mg kg^{-1} and ranged from 11-123% of total Cd levels (Table 8). The 300 mg kg^{-1} mean $Ca(NO_3)_2$-extractable Cd concentration was 179 mg kg^{-1} and ranged from 9-108% of total Cd levels (Table 9). Conder and Lanno (2000) found that $Ca(NO_3)_2$-extractable Cd levels in artificial soils were 0-59% of total Cd levels. The approximately 12-fold difference found for both $Ca(NO_3)_2$-extractable Cd concentrations expressed as percentages of total Cd in this study suggests that soil properties modulated extractable Cd levels.

Extraction techniques using weak salt solutions (< 1*M* $CaCl_2$ or $Ca(NO_3)_2$) estimate metal levels in soil pore water and readily dissolved metal adsorbed to soil components or in minerals with high water solubility. These forms of extractable metal are a more accurate measure of actual metal exposure than total metal levels (Lanno et al., 1999). This type of extraction technique has shown promise as a toxicity-related measure of bioavailability in soils (Conder and Lanno, 2000; Basta and Gradwohl, 2000; Posthuma et al., 1997; Sloan et al., 1997; Peijnenburg et al., 1997, 1999b; Weljte, 1998).

Because surrogate measures of bioavailability must be correlated with organismal responses (Lanno et al., 2002, in press), models were developed using both direct (internal concentrations) and indirect approaches (organismal

responses and weak electrolyte extractions) for assessing Cd bioavailability and toxicity. Models were developed for earthworm mortality, cocoon production, internal concentrations, extractable Cd, and bioaccumulation factors. Path analysis results for soils tested are listed in Tables 10 and 11. Simple correlation coefficient (*r*) values between pH, OC, clay, and extractable Cd concentrations are listed for comparison with path analysis results. Path analysis partitions each *r*-value into one direct effect (underlined, main diagonal positions) and two indirect effects (off diagonal positions). Significant direct effects are indicated by corresponding *p*-values for each model tested. Significant coefficient of determination (R^2) values and low uncorrelated (U) values indicate that the model explains most of the variation in each of the models tested. The model explains most of the variation observed in the 50 and 300 mg Cd kg^{-1} spiked soils for $Ca(NO_3)_2$-extractable Cd ($R^2 = 0.74$, $P < 0.01$ and $R^2 = 0.73$, $P < 0.01$, respectively). Simple correlation results (*r*-values) for both the 50 mg Cd kg^{-1} and 300 mg Cd kg^{-1} spiked soils indicate that that soil pH, OC, and clay strongly affected $Ca(NO_3)_2$-extractable Cd ($P < 0.05$). However, OC and clay do not retain significance in either model when partitioned into direct and indirect effects by path analysis. Simple correlation coefficients are significant due to indirect effects or intercorrelations of soil properties. Path analysis partitioning provides direct effects or causation of soil properties on $Ca(NO_3)_2$-extractable Cd. The path analysis partitioning of both models shows strong pH direct effects ($P <0.01$) on $Ca(NO_3)_2$-extractable Cd.

Regression analysis results for the 22 Cd spiked soils tested are listed in Tables 12 and 13. Backwards-stepwise regression was used to identify the critical soil properties that explain most of the variation of these parameters in 22 field soils. Backwards stepwise regression results indicate that pH and OC explained the variance among 50 mg Cd kg^{-1} $Ca(NO_3)_2$-extractable Cd concentrations (R^2 = 0.90, $P < 0.01$) and were highly predictive of available Cd in the soil (Table 12). Backwards stepwise regression results indicate that pH and clay explain the variance among 300 mg Cd kg^{-1} $Ca(NO_3)_2$-extractable Cd concentrations (R^2 = 0.86, $P < 0.01$) and were highly predictive of available Cd in the soil (Table 13).

Earthworm mortality

Cumulative mean (n = 66) earthworm mortality was 1.2%, which was < 10% in each of the 22 unspiked reference soils. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to Cd spiked soils (Figure 8). Adverse physiological responses to Cd exposure included dermal lesions, yellow secretions, and segmenting, typical of stress responses in *E. andrei* (Edwards and Bohlen, 1992). In soils with 100% mortality (Bernow B, Norge A and, Teller A), earthworms died during the first week of the experiment. Soils with low pH and OC resulted in many dead animals within the first day. Fifty percent mortality was observed for earthworms exposed to 50 mg Cd kg^{-1} spiked Norge A soils by day 2. This was the case for Norge A and Teller A soils, spiked with 300 mg Cd kg^{-1}, which had 99% and 83% mortality, respectively, by day 2. In these soils, all worms were dead by day 3. According to Spurgeon et al. (1994), this may

suggest that the main toxic effect of Cd was exerted by uptake across the body wall, rather than via dietary metal assimilation. Significant mortality was not observed for *Eisenia fetida* exposed to artificial soil spiked at a level of 300 mg Cd kg^{-1} (Spurgeon et al., 1994). The range of mortality observed in our study was the result of differences in Cd bioavailability due to Cd interactions with the soil properties, assuming similar behavior of earthworms in each soil.

Complete mortality (100%) was observed in 300 mg Cd kg^{-1} spiked soils with pH < 4.4. However, mean (SD) earthworm mortality was 1.7% (4.1) in the unspiked reference soils with pH < 4.4. The 12-fold difference of $Ca(NO_3)_2$-extractable Cd and complete mortality in the 300 mg Cd kg^{-1} soils with pH < 4.4 are evidence of the effects of soil properties modifying environmentally available Cd. Regression analysis established a relationship for $Ca(NO_3)_2$-extractable Cd and mortality in the 300 mg Cd kg^{-1} spiked soils (P = 0.04).

Results indicate that the model based on 50 mg Cd kg^{-1} did not adequately explain the variability in mortality (R^2 = 0.20) and could not be used with confidence to explain the variability in mortality (Table 10). In part, this finding may be attributed to the high percentage of survival observed in these soils. The low R^2 value may also suggest that factors other than soil pH, OC, and clay are needed to explain this model. Simple correlation results (r-values) indicate that soil pH significantly affected mortality (P < 0.05). However, pH did not retain significance when partitioned into direct and indirect effects by path analysis.

The model explains most of the variation observed in earthworm mortality in soils spiked with 300 mg Cd kg^{-1} (R^2 = 0.42, $P < 0.05$) (Table 11). Simple correlation results (*r*-values) indicate that soil pH and OC significantly affected mortality ($P <$ 0.05). However, OC did not retain significance when partitioned into direct and indirect effects by path analysis. Path analysis partitioning shows a causative or direct effect of pH on mortality ($P <0.05$).

Backwards-stepwise regression results reveal that pH was the only significant soil property modifying 50 mg Cd kg^{-1} soils for mortality (R^2 = 0.52, $P < 0.01$) (Table 12). Backwards-stepwise regression results reveal that pH, OC, and interactions of pH-clay and OC-clay are more important than clay alone for modifying 300 mg Cd kg^{-1} soils for mortality (R^2 = 0.83, $P < 0.01$) (Table 13). pH has been reported in regression models as the most important soil property modulating Cd uptake rates (Peijnenburg et al. 1999b, Janssen et al., 1997). Soils containing higher organic matter have a higher binding capacity for Cd (Adriano, 2001), potentially causing Cd to be unavailable for uptake by organisms.

Internal concentrations

The mean (SD) internal concentration of *E. andrei* exposed to unspiked reference soils was 8.5 mg Cd kg^{-1} (4.1) (Table 14). The mean Cd concentrations of earthworms exposed to 50 and 300 mg Cd kg^{-1} were 322 (267)

and 529 mg kg^{-1} (206), respectively (Table 14). Internal concentration differences of 17 (50 mg Cd kg^{-1} soils) and 12-fold (300 mg Cd kg^{-1}) in earthworms exposed to the same total soil content of Cd in 22 soils indicates that soil properties are modifying the uptake of Cd. This is consistent with the findings of Peijnenburg et al. (1999b), indicating that soil properties have a significant impact on Cd uptake by *E. andrei*.

Earthworms exposed to soils where 100% mortality occurred were removed from internal Cd concentration statistical models to avoid comparison of depurated and non-depurated worms because there may be differences in the soil content of the earthworm gut and differences in Cd uptake due the physiological effects of acutely toxic Cd exposure. The model explains most of the variation observed in internal concentrations based on worms exposed to 50 mg Cd kg^{-1} spiked soils ($R^2 = 0.43$, $P < 0.05$) (Table 10). Correlation results indicate that OC and clay significantly influenced internal concentrations ($P < 0.05$). Clay and OC do not remain significant when partitioned into causal or direct effects by path analysis. Path analysis partitioning shows non-significant causative or direct effects on internal Cd concentrations. As stated by Allen (2001), Cd uptake is dependent on the Cd concentration, binding capacity of the soil phase, and the physicochemical properties of the soil organic matter.

The model for internal concentrations of worms exposed to 300 mg Cd kg^{-1} soils explains most of the variation observed ($R^2 = 0.59$, $P < 0.01$) (Table 11).

Correlation results indicate that pH and clay strongly influenced internal concentrations ($P < 0.01$). Clay did not remain significant when partitioned into causal or direct effects by path analysis. Path analysis partitioning shows pH is a causative or direct effect ($P < 0.05$) on internal Cd concentrations. Previous research has indicated that pH is typically the main factor modulating metal solubility and bioavailability (McLean and Bledsoe, 1992; Peijnenburg et al., 1997, 1999b; Posthuma et al., 1997; Basta et al., 1993; Smit et al., 1998).

Backwards-stepwise regression results indicate that pH, OC, clay, and interactions of OC-clay contribute to explaining the variance for internal concentrations in soils spiked with 50 mg Cd kg^{-1} ($R^2 = 0.81$, $P < 0.01$) (Table 12). Backwards-stepwise regression results indicate that pH and clay contribute to explaining the variance among internal concentrations in soils spiked with 300 mg Cd kg^{-1} ($R^2 = 0.59$, $P < 0.01$) (Table 13). In a previous study, regression formulae suggest the most significant impact on Cd uptake is pH and that Cd is primarily taken in via the labile (extractable) soil fraction (Peijnenburg et al. 1999b). Regression analysis results for 50 mg Cd kg^{-1} soils established a relationship ($r^2 = 0.55$) for 0.5 M $Ca(NO_3)_2$-extractable Cd and internal Cd concentrations ($P < 0.01$) (Figure 9a). Regression analysis results for 300 mg Cd kg^{-1} soils established a relationship ($r^2 = 0.46$) for 0.5 M $Ca(NO_3)_2$-extractable Cd and internal Cd concentrations ($P < 0.01$) (Figure 9b).

Cd uptake does not necessarily mean toxicity will occur because earthworms may sequester metals avoiding physiological impact (Allen, 2001). The main detoxification pathway for Cd is thought to be sequestration within inorganic matrices or binding to organic ligands (Spurgeon and Hopkin, 1999). The main site of storage is the chloragogenous tissue that contains granules of sulfur-donating ligands in the form of residues of metallothionein type proteins (Morgan and Morgan, 1989). These proteins are involved in binding type B metals such as Cd (Spurgeon and Hopkin, 1999). Adverse effects can be expected only when the capacity of detoxification mechanisms is exceeded, occurring at a very high internal concentration when metal is slowly sequestered (Lock, 2001). Resulting mortalities in many of the soils tested suggests that detoxification mechanisms were exceeded. Regression analysis found the relationship between internal concentrations and mortality was non-significant ($P > 0.12$) for 50 and 300 mg Cd kg^{-1}, which is consistent with previous data (Lock, 2000).

Cocoon production

Cocoon production of *E. andrei* decreased with increasing internal concentrations (Figure 10). Similar results were observed for *Eisenia fetida* cocoon production when exposed to Cd spiked artificial soils and one sandy and loamy field soil (Lock et al., 2001). The model explains most of the variation observed in the 50 mg Cd kg^{-1} soils for cocoon production ($R^2 = 0.38$, $P < 0.05$). Simple correlation results reveal that OC influenced cocoon production ($P < 0.01$) (Table 10). Path analysis partitioning indicated a causative or direct effect of OC on cocoon

production ($P < 0.05$). The model explains most of the variation observed in the 300 mg Cd kg^{-1} soils for cocoon production ($R^2 = 0.51$, $P < 0.01$). Simple correlation results reveal that pH and OC influenced cocoon production ($P < 0.05$) (Table 11). Path analysis partitioning indicated a causative or direct effect of OC on cocoon production ($P < 0.05$). The natural habitats for *E. andrei* are those of very high organic matter such as manure or compost piles (ASTM, 1997), which may result in the direct effect of OC on cocoon production. However, OC was not removed from statistical models because this may result in inaccurate conclusions of significant effects on cocoon production.

Backwards-stepwise regression results indicate that OC contributes most to explaining the variance among cocoon production for 50 mg Cd kg^{-1} soils ($R^2 = 0.36$, $P < 0.01$) (Table 12). Backwards-stepwise regression results indicate that pH, OC, clay, and pH-clay and pH-OC interactions contribute to explaining the variance among cocoon production for 300 mg Cd kg^{-1} model ($R^2 = 0.81$, $P < 0.01$) (Table 13). This, however, conflicts with the regression results for 50 mg Cd kg^{-1} indicating OC contributed more than pH or clay to cocoon production.

Biota-soil accumulation factors

Biota-soil accumulation factors were developed for this study based on total Cd concentrations ($BSAF_{Total}$) and weak electrolyte extractions ($BSAF_{Ca(NO3)2}$). Biota-soil accumulation factors represent the ratio of internal Cd concentrations in exposed earthworms to concentrations in the exposure matrix. Calculated $BSAF_{Total}$ and $BSAF_{Ca(NO3)2}$ values are listed in Table 15. Calculated biota-soil

accumulation factors for 50 mg Cd kg^{-1} soils ranged approximately 20-fold from 1.5 – 30 for $BSAF_{Total}$ and from 2.1 – 32 for $BSAF_{Ca(NO3)2}$. Calculated biota-soil accumulation factors for 300 mg Cd kg^{-1} soil ranged from approximately 1.0 – 3.0 for $BSAF_{Total}$ and from 1.8 – 13 for $BSAF_{Ca(NO3)2}$. Because BSAFs are assumed to be independent of soil Cd concentrations, they are often used to assess the effect of soil properties on bioavailability (Peijnenburg et al., 1999a, 1999b; Janssen, 1997). Results show $BSAF_{Ca(NO3)2}$ values decreased with increasing extractable Cd concentrations (Figure 11). Lock and Janssen (2001) showed similar results for BSAFs based on total Cd in soil.

Significant coefficient of determination (R^2) values and low uncorrelated (U) values indicate that the path analysis model explains the variation in both Cd $BSAF_{Ca(NO3)2}$ models (Tables 10 and 11). Simple correlation results indicated a significance of pH on the 50 mg Cd kg^{-1} $BSAF_{Ca(NO3)2}$ model ($P < 0.05$). Path analysis partitioning indicates that pH and clay are significant causative or direct effects on the 50 mg Cd kg^{-1} $BSAF_{Ca(NO3)2}$ model ($P < 0.05$). Simple correlation results indicated a significance of pH on the 300 mg Cd kg^{-1} $BSAF_{Ca(NO3)2}$ model ($P < 0.01$). Path analysis partitioning indicates that pH remains the significant causative or direct effect on the 300 mg Cd kg^{-1} $BSAF_{Ca(NO3)2}$ model ($P < 0.01$).

Backwards-stepwise regression results for 50 mg Cd kg^{-1} soils indicate that OC and clay, in addition to a pH-OC interaction, are significant in explaining the variance among $BSAF_{Ca(NO3)2}$ values ($R^2 = 0.60$, $P < 0.01$) (Table 12).

Backwards-stepwise regression results for 300 mg Cd kg^{-1} suggest pH and OC, in addition to a pH-OC interaction, are significant in explaining the variance among $BSAF_{Ca(NO3)2}$ ($R^2 = 0.88$, $P < 0.01$) (Table 13). Peijnenburg, et al. (1999b) showed that the variance in bioaccumulation factors for Cd was best explained by soil pH. Previous earthworm bioaccumulation factor models have shown that low pH increases the uptake of Cd while high soil organic matter reduces bioavailability (Corp and Morgan, 1991; Ma, 1982). Soil organic matter was shown to be associated with bioaccumulation of Cd in earthworms (Morgan and Morgan, 1988). Regression models developed in this study are consistent with these findings. However, path analysis models generated indicate that pH is significant for bioaccumulation factors based on total Cd and pH and OC are significant for bioaccumulation factors based on $Ca(NO_3)_2$-extractable Cd.

Bioaccumulation factors are frequently used to assess the effect of soil-modifying factors on the bioavailability of metals in soils (Janssen et al., 1997; Peijnenburg et al., 1999a, 1999b). However, there is much debate about the use of bioaccumulation factors to assess the bioavailability of metals. Some authors report that bioaccumulation factors should be questioned because they tend to decrease with increasing metal concentrations, indicating there is no relationship between the internal concentration and the bioavailable concentration (Lock, 2000). Others state that bioaccumulation factors are more appropriate than body concentrations for normalization among field soils (Janssen, 1997). Bioaccumulation factors are usually normalized to total metal concentrations in soil. Peijneburg et al. (1999), suggests bioaccumulation factors should be based

on bioavailable concentrations in the soil. In this study, bioaccumulation factors based on $Ca(NO_3)_2$-extractable Cd were found to increase with increasing concentrations of available Cd. It is assumed that bioaccumulation occurs when BSAFs are greater than one (Lock, 2001). Bioaccumulation factors based on total and available Cd in this study were consistently greater than unity in all soils. However, in soils with the largest BSAF values, little or no mortality was observed suggesting that biota-soil accumulation factors are poor indicators of adverse effects of Cd to earthworms ($P > 0.10$).

Empirical formulas developed may be useful for predicting the potential environmental risks of Cd in soil. Path analysis models proved useful for providing a quantitative causal influence of Cd bioavailability and toxicity to earthworms. pH was the most important soil property modifying the bioavailability and toxicity of Cd. Conclusions of our study support the use of weak-electrolyte extractions as a surrogate measure of bioavailability. The absence of a significant relationship found for internal concentrations and mortality suggests that internal concentrations may not prove useful as indicators of adverse effects of Cd toxicity and bioavailability to earthworms. Biota-soil accumulation factors in this study were deemed as poor indicators of environmental risk of Cd toxicity. Furthermore, the decrease in $BSAF_{Ca(NO3)2}$ with increasing available Cd concentrations indicates that BSAFs should not be used assess the influence of soil properties on Cd bioavailability.

Acknowledgements

We would like to thank Derrick Chappell for assistance with bioassays. The research in this manuscript has been funded by the U.S. Environmental Protection Agency, Office of Research and Development, National Center for Exposure Assessment. Although the research described in this paper has been funded by the U.S. EPA to Roman Lanno and Dr. Nicholas Basta, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

Adriano, DC. 2001. *Trace elements in terrestrial environments.* Springer, New York, USA.

American Society for Testing and Materials. 1997. Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with Lumbricid earthworm *Eisenia fetida*, standard E 1676-97. In: Annual Book of ASTM Standards, pp. 1056-1074.

Basta NT, Pantone DJ, Tabatabai MA. 1993. Path analysis of heavy metal adsorption by soil. *Agron. J.* 85:1054-1057.

Basta NT, Gradwohl R. 2000. Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soil by a sequential extraction procedure. *Journal of Soil Contamination.* 9:149-164.

Beyer WN, Hensler G, Moore, J. 1987. Relation of pH and other soil variables to concentrations of Pb, Cu, Zn, Cd, and Se in earthworms. *Pedobiologia.* 30:167-172.

Conder JM, Lanno RP. 2000. Evaluation of cadmium, lead, and zinc to *Eisenia fetida. Chemosphere.* 41:1659-1668.

Corp N, Morgan AJ. 1991. Accumulation of heavy metals from polluted soils by the earthworm, *Lumbricus rubellus*: can laboratory exposure of 'control' worms reduce biomonitoring problems? *Environ. Poll.* 74:39-52.

Edelman WMT, Van Beersum I, Jans T. 1983. Uptake of Cd, Zn, Pb, and Cu by earthworms near a zinc smelting complex: influence of soil pH and organic matter. *Bull. Environ. Contam. Toxicol.* 30:424-427.

Edwards CA, Bohlen PJ. 1992. The effects of toxic chemicals on earthworms. *Rev. Environ. Contam. Toxicol.* 125:23-99.

Gee GW, Bauder JW. 1986. Particle-size analysis. In A. Klute, ed., *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods,* 2nd ed. Agronomy Monograph 9. Soil Science Society of America, Madison, WI, USA, pp. 383-411.

Heanes DL. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Commun. In Soil Sci. Plant Analysis.* 15:1191-1213.

Hendershot WH, Duquette M. 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Sci. Soc. Am. J.* 50:605-608.

Janssen RPT, Posthuma L, Baerselman R, Den Hollander HA, Van Veen RPM, Peijnenburg WJGM. 1997. Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal accumulation in earthworms. *Environ. Toxicol. Chem.* 16:2479-2488.

Lanno R. Ed. 2002 (In press). *Contaminated soils: From soil-chemical interactions to ecosystem management. Proceedings of Workshop on Assessing Contaminated Soils, Pellston, MI, 23-27 September 1998.* Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, USA.

Lanno RP, Conder JM, Seals L. 1999. Critical limits for heavy metals in soils and surface waters. *Workshop on effects-based approaches for heavy metals.* Federal Environmental Agency (Umweltbundesamt), Berlin, October.

Lanno RP, McCarty LS. 1997. Earthworm bioassays: Adopting techniques from aquatic toxicology. *Soil Biol. Biochem.* 29:693-697.

Lock K, Janssen CR. 2001. Zinc and cadmium body burdens in terrestrial Oligochaetes: use and significance in environmental risk assessment. *Environ. Toxicol. Chem.* 20:2067-2072.

Lock K, Janssen CR, De Coen WM. 2000. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to *Enchytraeus albidus*. *Environ. Toxicol. Chem.* 19:2666-2671.

Loehlin JC. 1987. *Latent variable models: an introduction to factor, path, and structural analysis.* Lawrence, Hillsdale, New Jersey, USA.

Ma WC. 1982. The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. *Pedobiologia* 24:109-119.

McBride MB. 1989. Reactions controlling heavy metal solubility in soils. *Adv. Soil Sci.* 10:1-56.

McLean LE, Bledsoe BE. 1992. Ground water issue: Behavior of metals in soils. EPA/540/S-92/018. U.S. Environmental Protection Agency, Washington, DC.

Morgan JE, Norey CG, Morgan AJ, Kay J. 1989. A comparison of the cadmium-binding proteins isolated from the posterior alimentary canal of the earthworm *Dendrodrilus rubidus* and *Lumbricus rubellus*. *Comp. Biochem. Physiol.* 92:15-21.

Morgan ME, Morgan AJ. 1988. Earthworms as biological monitors of Cd, Cu, Pb, and Zn in metalliferous soils. *Environ. Pollut.* 54:123-138.

Morgan AJ, Morris B. 1982. The accumulation and intracellular compartmentation of cadmium, lead, zinc, and calcium in two earthworm species (*Dendrobaena rubida* and *Lumbricus rubellus*) living in highly contaminated soil. *Histochem.* 75:269-285.

Peijnenburg WJGM, Posthuma L, Zweers PGPC, Baerselman R, de Groot AC, van Veen RPM, Jager T. 1999a. Prediction of metal bioavailability in Dutch field soils for the Oligochaeta *Enchytraeus crypticus*. *Ecotoxicol. Environ. Safety* 43:170-186.

Peijnenburg WJGM, Baerselman R, de Groot AC, Jager T, Posthuma L, Van Veen RPM. 1999b. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. *Ecotoxicol. Environ. Saf.* 44:294-310.

Peijnenburg WJGM, Posthuma L, Eijsackers HJP, Allen HE. 1997. A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicol. Environ. Safety* 37:163-172.

Polemio M, Rhoades JD. 1977. Determining cation exchange capacity: a new procedure for calcareous and gypsiferous soils. *Soil Sci. Soc. Am. J.* 41:524-528.

Posthuma L, Baerselman R, Van Veen RPM, Dirven-Van Breemen EM. 1997. Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. *Ecotoxicol. Environ. Safety* 38:108-121.

Rieuwerts JS, Thornton I, Farago ME, Ashmore, MR. 1998. Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chem. Spec. Bioavail.* 10:61-75.

Sloan JJ, Dowdy RH, Dolan MS, Linden DR. 1997. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. *J. Environ. Qual.* 26:966-974.

Smit CE, Posthuma L, Folkerts AJ, Baerselman R, Dirven-Van Breemen EM, Bogte J, Van Beelen P. 1998. Heavy metal behavior in OECD artificial soil, Budel gradient soils and experimental field plot soil-methodological aspects and soil chemical characterization of contamination. In: Posthuma L, Van Gestel CAM, Smit CE, Bakker DJ, Vonk JW. eds, *Validation of Toxicity Data and Risk Limits for Soils: Final Report.* National Institute of Public Health and Environment (RIVM), Bilthoven, The Netherlands, p. 230.

Spurgeon DJ, Hopkin SP. 1999. Tolerance to zinc in populations of the earthworm *Lumbricus rubellus* from uncontaminated and metal-contaminated ecosystems. *Arch. Environ. Contam. Toxicol.* 37:332-337.

Spurgeon DJ, Hopkin SP, Jones DT. 1994. Effects of Cd, Cu, Pb, and Zn on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ. Poll.* 84:123-130.

Thomas GW. 1996. Soil pH and soil acidity. In Sparks DL, ed, *Methods of Soil Analysis.* Part 3 Chemical Methods-SSSA Book series no. 5. Soil Science Society of America, Madison, WI, pp. 475-490.

U.S. Environmental Protection Agency. 1994. Method 3051: Microwave assisted acid digestion of sediment, sludges, soils, and oils.

U.S. Environmental Protection Agency. 2000. Ecological soil screening level guidance. Draft Report. Office of Emergency and Remedial Response. Washington, D.C.

Van Gestel CAM, Dirven-van Breeman EM, Baerselman R. 1993. Accumulation and elimination of cadmium, chromium, and zinc and the effects on growth and reproduction of *Eisenia andrei* (Oligochaeta, Annelida). *Sci. Total Environ. Supplement* 585-597.

Weljte L. 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb, and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. *Chemosphere* 36:2643-2660.

Williams WA, Jones MB, Demment MW. 1990. A concise table for path analysis statistics. *Agron. J.* 82:1022-1024.

Table 7. Properties of Soils

Soil	Horizon	Unspiked Soil pH[a]	Cd 50 mg/kg spiked soil pH[a]	Cd 300 mg/kg spiked soil pH[a]	CEC cmol/kg[b]	OC	Sand %	Clay	Silt	Class
Bernow	B	4.9	4.2	4.1	6.74	0.30	58.8	26.3	17.5	Sandy Clay Loam
Canisteo	A	7.5	7.8	7.9	30.5	3.00	31.3	38.8	51.3	Silt Loam
Dennis	A	5.6	4.8	4.8	9.77	1.90	37.5	23.8	40.0	Loam
Dennis	B	6.1	5.7	5.5	14.6	0.80	21.3	45.0	40.6	Clay Loam
Doughtery	A	5.3	4.7	4.7	3.33	1.20	75.0	11.3	21.3	Loan Sand
Hanlon	A	7.4	6.5	6.6	16.3	1.60	63.8	17.5	23.8	Sandy Loam
Kirkland	A	5.6	4.8	4.7	14.0	1.45	12.5	31.3	57.5	Silty Clay Loam
Luton	A	7.1	7.1	6.8	32.4	2.00	2.50	71.3	38.8	Clay
Mansic	A	7.8	7.3	7.6	16.5	1.50	33.8	30.0	43.8	Loam
Mansic	B	8.0	7.8	7.6	11.7	0.53	30.0	35.0	42.5	Clay Loam
Norge	A	4.0	3.8	3.9	4.57	1.20	36.9	17.5	45.6	Silt Loam
Osage	A	6.6	6.5	6.1	28.3	2.60	13.8	55.7	53.8	Silty Clay Loam
Osage	B	6.8	6.1	5.8	27.5	2.00	11.3	61.3	47.5	Silty Clay
Pond Creek	A	5.2	4.6	4.4	10.7	1.90	16.3	28.8	62.5	Silt Loam
Pond Creek	B	6.0	5.8	5.7	12.5	0.80	18.8	32.5	48.8	Silty Clay Loam
Pratt	A	6.5	5.5	5.2	4.40	0.90	90.0	5.00	3.80	Silt
Pratt	B	6.4	5.5	5.2	3.40	0.50	92.5	6.25	1.30	Silt
Richfield	B	7.7	6.7	6.8	22.3	1.10	11.3	41.3	51.3	Silty Clay Loam
Summit	A	7.2	7.0	7.1	29.4	2.40	17.5	45.7	53.8	Silty Clay Loam
Summit	B	7.1	6.9	6.6	27.6	1.25	10.0	56.8	48.8	Silty Clay
Taloka	A	5.1	4.4	4.5	4.85	1.20	20.0	11.3	58.8	Silt Loam
Teller	A	4.5	3.9	3.9	3.01	0.85	66.9	10.0	23.8	Silt Loam
MINIMUM		4.0	3.8	3.9	3.01	0.30	2.50	5.00	1.30	
MAXIMUM		8.0	7.8	7.9	32.4	3.00	92.5	71.3	62.5	
MEAN		6.3	5.8	5.7	15.2	1.41	35.1	32.0	40.0	
MEDIAN		6.5	5.7	5.6	13.3	1.25	25.7	30.7	45.0	

[a] pH determined by 1:1 soil:water
[b] Cation Exchange Capacity measured using 0.1 M $BaCl_2$ for non-calcareous soils; 1 M NaOAc, pH 8.2 for calcareous soils.

Table 8. Total and $Ca(NO_3)_2$-extractable Cd 50 mg/kg soil concentrations.

Soil	Horizon	$Ca(NO_3)_2$-extractable Cd[1] mg kg^{-1}	%RSD	%[2]	Total Cd[3] mg kg^{-1}	%RSD
Bernow	B	45.3	3.76	108	41.8	0.59
Canisteo	A	7.18	33.7	17.2	41.8	2.35
Dennis	A	36.4	3.67	75.1	48.5	0.32
Dennis	B	27.4	2.59	59.0	46.4	1.04
Dougherty	A	36.8	4.49	80.0	46.1	4.01
Hanlon	A	32.9	6.29	74.2	44.4	1.67
Kirkland	A	15.2	11.6	31.6	48.0	0.44
Luton	A	42.4	9.39	79.7	53.2	6.97
Mansic	A	37.6	0.95	77.6	48.5	0.41
Mansic	B	12.6	2.62	25.0	50.5	0.42
Norge	A	6.46	2.61	14.3	45.2	1.31
Osage	A	4.98	6.24	10.5	47.3	2.21
Osage	B	19.9	4.81	39.9	49.9	2.89
Pond Creek	A	23.3	2.04	48.2	48.3	1.32
Pond Creek	B	33.7	8.72	69.9	48.3	0.44
Pratt	A	33.1	1.61	71.8	46.1	2.82
Pratt	B	43.8	3.44	93.9	46.6	2.53
Richfield	B	40.4	25.9	75.0	53.9	6.83
Summit	A	49.3	4.73	123	40.2	0.46
Summit	B	19.6	5.46	44.6	43.9	1.19
Taloka	A	8.26	5.19	16.5	50.0	0.62
Teller	A	20.8	2.14	38.6	53.9	1.64
MINIMUM		4.98	0.95	10.5	40.2	0.32
MAXIMUM		49.3	33.7	123	53.9	6.97
MEAN		27.2	6.90	57.9	47.4	1.93
MEDIAN		30.2	4.61	64.4	47.7	1.32

[1] Extracted using 0.5 M $Ca(NO_3)_2$, mean (n=3)
[2] Percent of total metal that was $Ca(NO_3)_2$-extractable
[3] Extracted according to EPA Method 3051 and measured by ICP-AES, mean (n=2)

Table 9. Total and $Ca(NO_3)_2$-extractable Cd 300 mg/kg soil concentrations.

Soil	Horizon	$Ca(NO_3)_2$-extractable Cd[1] mg kg^{-1}	%RSD	%[2]	Total Cd[3] mg kg^{-1}	%RSD
Bernow	B	217	0.97	80.5	270	2.69
Canisteo	A	43.6	1.85	14.2	306	0.79
Dennis	A	235	2.06	75.2	312	0.12
Dennis	B	198	3.07	62.8	315	0.52
Dougherty	A	253	12.3	84.2	300	1.05
Hanlon	A	127	3.45	43.5	293	2.07
Kirkland	A	254	1.25	83.6	303	0.65
Luton	A	118	4.59	36.1	326	1.27
Mansic	A	54.6	3.35	18.8	291	0.44
Mansic	B	26.2	2.35	8.50	309	0.21
Norge	A	232	3.97	80.6	288	1.02
Osage	A	136	4.62	47.0	290	2.51
Osage	B	169	2.14	55.2	306	1.42
Pond Creek	A	200	0.89	70.2	285	0.34
Pond Creek	B	256	7.71	83.6	306	3.19
Pratt	A	221	0.77	71.6	308	1.34
Pratt	B	351	2.51	108	324	1.62
Richfield	B	140	8.40	50.2	278	9.63
Summit	A	74.8	4.02	23.8	314	0.68
Summit	B	154	1.91	50.0	309	0.33
Taloka	A	242	1.32	76.9	315	0.86
Teller	A	230	5.10	78.2	295	1.37
MINIMUM		26.24	0.77	8.50	269.9	0.12
MAXIMUM		351	12.3	108	325.8	9.63
MEAN		179	3.57	59.2	301.9	1.55
MEDIAN		199	2.79	66.5	305.8	1.03

[1] Extracted using 0.5 M $Ca(NO_3)_2$, mean (n=3)
[2] Percent of total metal that was $Ca(NO_3)_2$-extractable
[3] Extracted according to EPA Method 3051 and measured by ICP-AES, mean (n=2)

Table 10. Path analysis direct effects (diagonal, underlined) and indirect effects (off diagonal) of soil pH, organic carbon (% OC), and clay (mmol kg^{-1}) on extractable Cd 50 mg/kg concentrations and *Eisenia andrei* after 28-day exposure to Cd 50 mg/kg.

Response		pH	OC	Clay	r	R^2	U
$Ca(NO_3)_2$	pH	-0.72**	-0.09	-0.03	-0.83**	0.74**	0.51
Extractable Pb	OC	-0.27	-0.23	-0.02	-0.53*		
(mg/kg)	Clay	-0.42	-0.11	-0.05	-0.58**		
% Mortality	pH	-0.44	0.01	-0.01	-0.45*	0.20	0.89
	OC	-0.17	0.02	-0.01	-0.16		
	Clay	-0.26	0.01	-0.02	-0.27		
Internal	pH	-0.10	-0.11	-0.21	-0.42	0.43*	0.76
concentration[a]	OC	-0.04	-0.31	-0.18	-0.52*		
(mg/kg dry wt.)	Clay	-0.05	-0.14	-0.39	-0.58**		
Cocoon	pH	-0.17	0.24	0.06	0.12	0.38*	0.79
Production[b]	OC	-0.07	0.62*	0.05	0.60**		
	Clay	-0.10	0.30	0.10	0.30		
$BSAF_{Ca(NO3)2}$[a]	pH	0.81**	0.03	-0.32	0.51*	0.51**	0.70
	OC	0.29	0.09	-0.28	0.09		
	Clay	0.43	0.04	-0.61*	-0.14		

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Soils with 100% mortality removed from model

[b] Cumulative (mean of 3 replicates per soil-Cd combination) cocoons

Table 11. Path analysis direct effects (diagonal, underlined) and indirect effects (off diagonal) of soil pH, organic carbon (% OC), and clay (mmol kg^{-1}) on extractable Cd 300 mg/kg concentrations and *Eisenia andrei* after 28-day exposure to Cd 300 mg/kg.

Response		pH	OC	Clay	r	R^2	U
$Ca(NO_3)_2$ extractable Pb (mg/kg)	pH	-0.74**	-0.06	-0.04	-0.84**	0.73**	0.52
	OC	-0.29	-0.15	-0.03	-0.47*		
	Clay	-0.40	-0.07	-0.07	-0.54*		
% Mortality	pH	-0.53*	-0.09	0.01	-0.61**	0.42*	0.76
	OC	-0.21	-0.23	0.01	-0.43*		
	Clay	-0.29	-0.11	0.02	-0.38		
Internal concentration[a] (mg/kg dry wt.)	pH	-0.55*	0.03	-0.18	-0.70**	0.59**	0.64
	OC	-0.15	0.13	-0.18	-0.20		
	Clay	-0.26	0.06	-0.39	-0.59**		
Cocoon production[b]	pH	0.35	0.20	-0.01	0.54*	0.51**	0.70
	OC	0.14	0.51*	-0.01	0.64**		
	Clay	0.19	0.25	-0.02	0.42		
$BSAF_{Ca(NO3)2}$[a]	pH	0.82**	0.04	-0.17	0.68**	0.56**	0.66
	OC	0.22	0.14	-0.17	0.19		
	Clay	0.38	0.06	-0.37	0.07		

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Soils with 100% mortality removed from model

[b] Cumulative (mean of 3 replicates per soil-Cd combination) cocoons

Table 12. Multiple regression formulae describing the quantitative relationship between soil properties, *Eisenia andrei* after 28-day exposure to Cd 50 mg/kg, and extractable Cd 50 mg/kg concentrations.

Response	Regression equation obtained[a]	Statistics
$Ca(NO_3)_2$-extractable Cd (mg/kg)	$y = -34.1 + 38.8(pH) - 24.6(OC) - 4.13(pH)^2 + 6.53(OC)^2$	$R^2 = 0.90$, n = 22, $P < 0.01$
% Mortality	$y = 325.9 - 107.3(pH) + 8.628(pH)^2$	$R^2 = 0.52$, n = 22, $P < 0.01$
Internal concentration[b] (mg/kg dry wt.)	$y = -2128 + 1255(pH) - 875.1(OC) - 18.31(Clay) - 107.9(pH)^2 + 153.4(OC)^2 + 8.141(OC*clay)$	$R^2 = 0.81$, n = 21, $P < 0.01$
Cocoon production[b,c]	$y = -5.33 + 8.37(OC)$	$R^2 = 0.36$, n = 21, $P < 0.01$
$BSAF_{Ca(NO3)2}$[b]	$y = 18.40 - 20.17(OC) - 0.003(Clay)^2 + 3.224(pH*OC)$	$R^2 = 0.60$, n = 21, $P < 0.01$

[a] All variables in the models are significant ($P < 0.1$)
[b] Soils with 100% mortality were removed from model
[c] Cumulative (mean of 3 replicates per soil-Cd combination) cocoons

Table 13. Multiple regression formulae describing the quantitative relationship between soil properties, *Eisenia andrei* after 28-day exposure to Cd 300 mg/kg, and extractable Cd 300 mg/kg concentrations.

Response	Regression equation obtained[a]	Statistics
$Ca(NO_3)_2$-extractable Cd (mg/kg)	$y = -303.8 + 234.3(pH) - 1.173(Clay) - 23.97(pH)^2$	$R^2 = 0.86$, n = 22, $P < 0.01$
% Mortality	$y = 712 - 215(pH) - 60.7(OC) + 17.6(pH)^2 - 0.21(pH*Clay) + 1.20(OC*Clay)$	$R^2 = 0.83$, n = 22, $P < 0.01$
Internal concentration[b] (mg/kg dry wt.)	$y = 953 - 3.51(Clay) - 7.38(pH)^2$	$R^2 = 0.59$, n = 19, $P < 0.01$
Cocoon Production[b,c]	$y = 53.3 - 18.5(pH) - 10.1(OC) + 1.62(pH)^2 + 0.01(Clay)^2 + 2.15(pH*OC) - 0.06(pH*Clay)$	$R^2 = 0.81$, n = 19, $P < 0.01$
$BSAF_{Ca(NO3)2}$[b]	$y = 31.36 - 10.91(pH) + 1.173(pH)^2 + 2.686(OC)^2 - 1.366(pH*OC)$	$R^2 = 0.88$, n = 19, $P < 0.01$

[a] All variables left in the models are significant ($P < 0.1$)
[b] Soils with 100% mortality were removed from model
[c] Cumulative (mean of 3 replicates per soil-Cd combination) cocoons

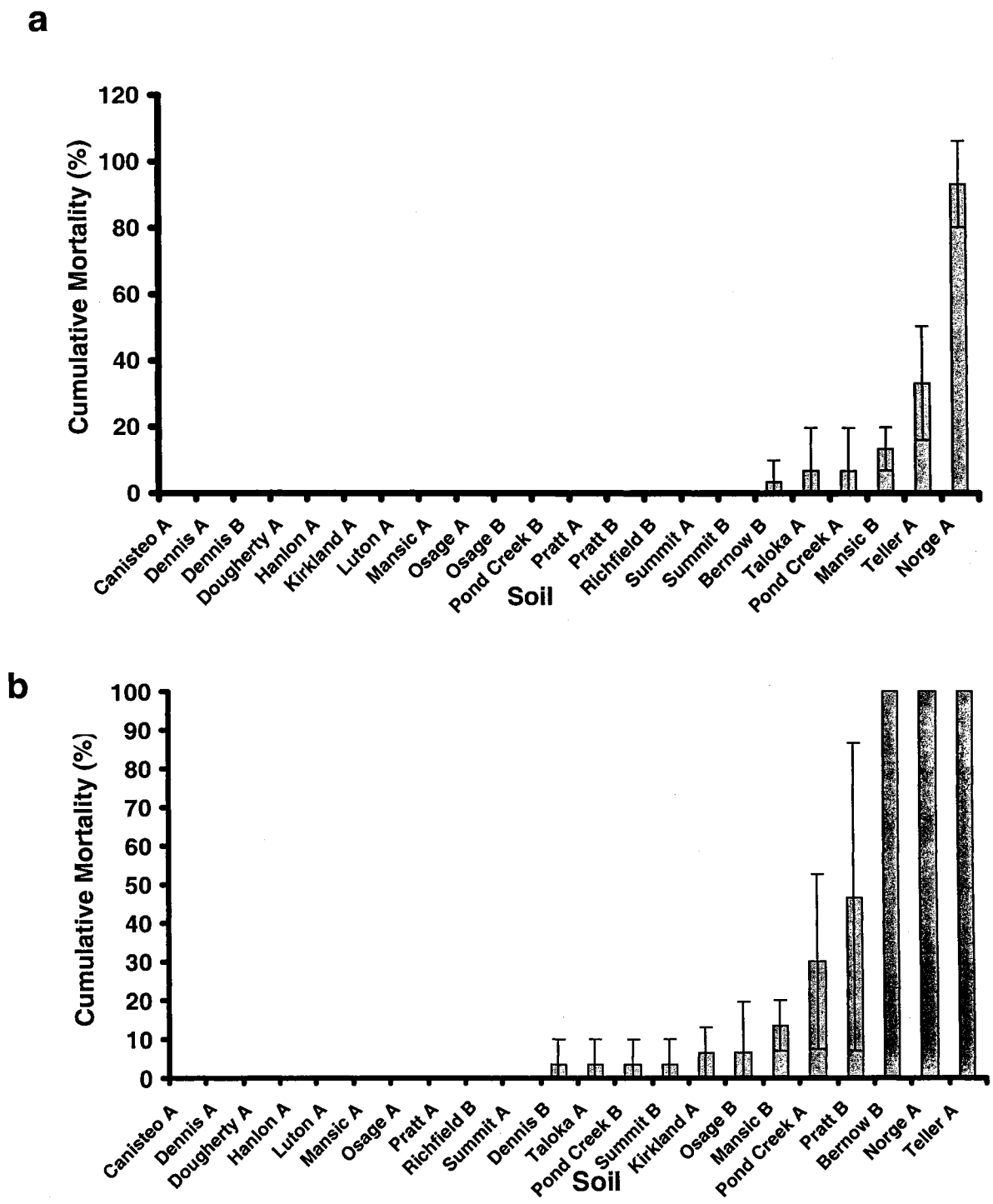

Figure 8. Cumulative mortality (mean of 3 replicates, ± 95% CI) of *Eisenia andrei* exposed to (a) 50 mg Cd/kg and (b) 300 mg Cd/kg spiked soils for

Table 14. Internal concentrations of Eisenia andrei exposed to unspiked and Cd spiked soils (mean, n = 3).

Soil	Horizon	Unspiked soil earthworm concentration[1] mg kg^{-1}	%RSD	Cd 50 mg/kg spiked soil earthworm concentration[2] mg kg^{-1}	%RSD	Cd 300 mg/kg spiked soil earthworm concentration[2] mg kg^{-1}	%RSD
Bernow	B	BDL	0.00	527	35.4	541	7.10
Canisteo	A	6.66	49.4	173	35.7	441	17.5
Dennis	A	8.77	49.8	321	12.1	598	16.3
Dennis	B	9.83	20.2	251	33.0	450	33.8
Dougherty	A	9.27	26.7	325	52.9	908	25.1
Hanlon	A	BDL	0.00	148	22.3	466	15.5
Kirkland	A	8.66	7.07	198	12.6	538	10.4
Luton	A	13.7	47.5	333	22.7	339	29.3
Mansic	A	10.2	51.5	286	19.3	318	19.8
Mansic	B	9.49	23.8	135	18.4	331	29.8
Norge	A	7.91	17.6	150	14.6	77.4	11.4
Osage	A	9.74	29.7	85.8	29.1	705	36.8
Osage	B	12.5	43.9	169	17.8	383	33.2
Pond Creek	A	5.59	6.13	195	16.9	674	10.0
Pond Creek	B	1.73	173	71.3	17.4	867	21.4
Pratt	A	7.71	31.9	266	10.5	846	10.8
Pratt	B	7.18	18.8	719	42.3	641	6.61
Richfield	B	7.87	12.3	788	38.6	434	20.7
Summit	A	5.51	51.7	1190	31.8	496	11.7
Summit	B	7.33	20.1	241	12.8	444	28.8
Taloka	A	11.8	39.8	267	18.3	765	6.93
Teller	A	1.49	173	231	30.0	366	22.3
MINIMUM		1.73	6.13	71.3	10.5	77.4	6.61
MAXIMUM		13.7	173	1190	52.9	908	36.8
MEAN		8.50	37.9	322	24.7	529	19.3
MEDIAN		8.66	29.7	246	20.8	481	18.6

[1] Cd concentration in digests of worms exposed to reference (unspiked) soils, measured by FAAS (limit of detection 10 µg/L)
[2] Cd concentration in digests of worms exposed to 50 and 300 mg/kg Cd spiked soils, measured by FAAS (limit of detection 10 µg/L)

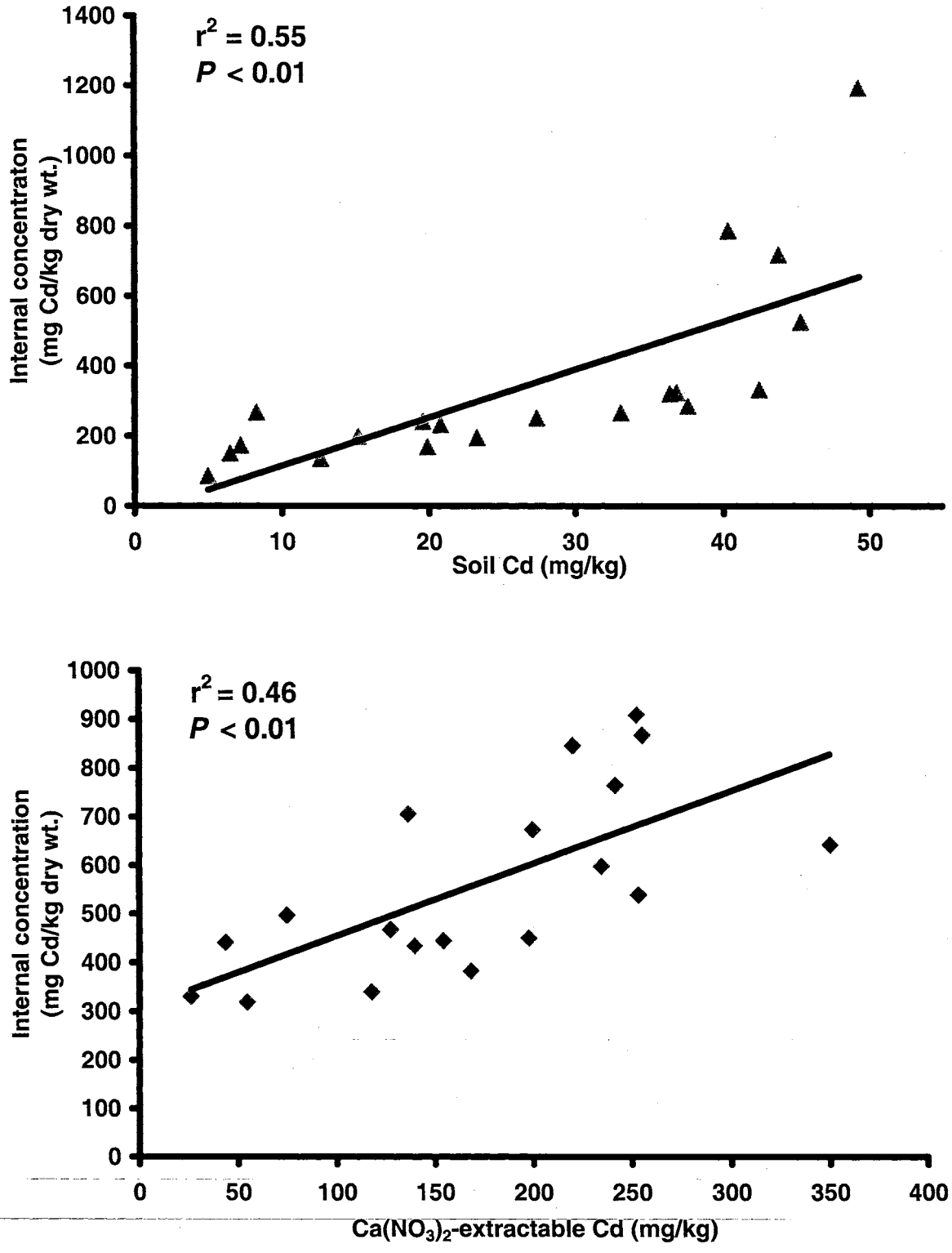

Figure 9. Cd concentrations (mean, n = 3 per soil) of *Eisenia andrei* exposed to (a) 50 mg Cd/kg and (b) 300 mg Cd/kg spiked soils versus $Ca(NO_3)_2$-extractable soil Cd concentrations.

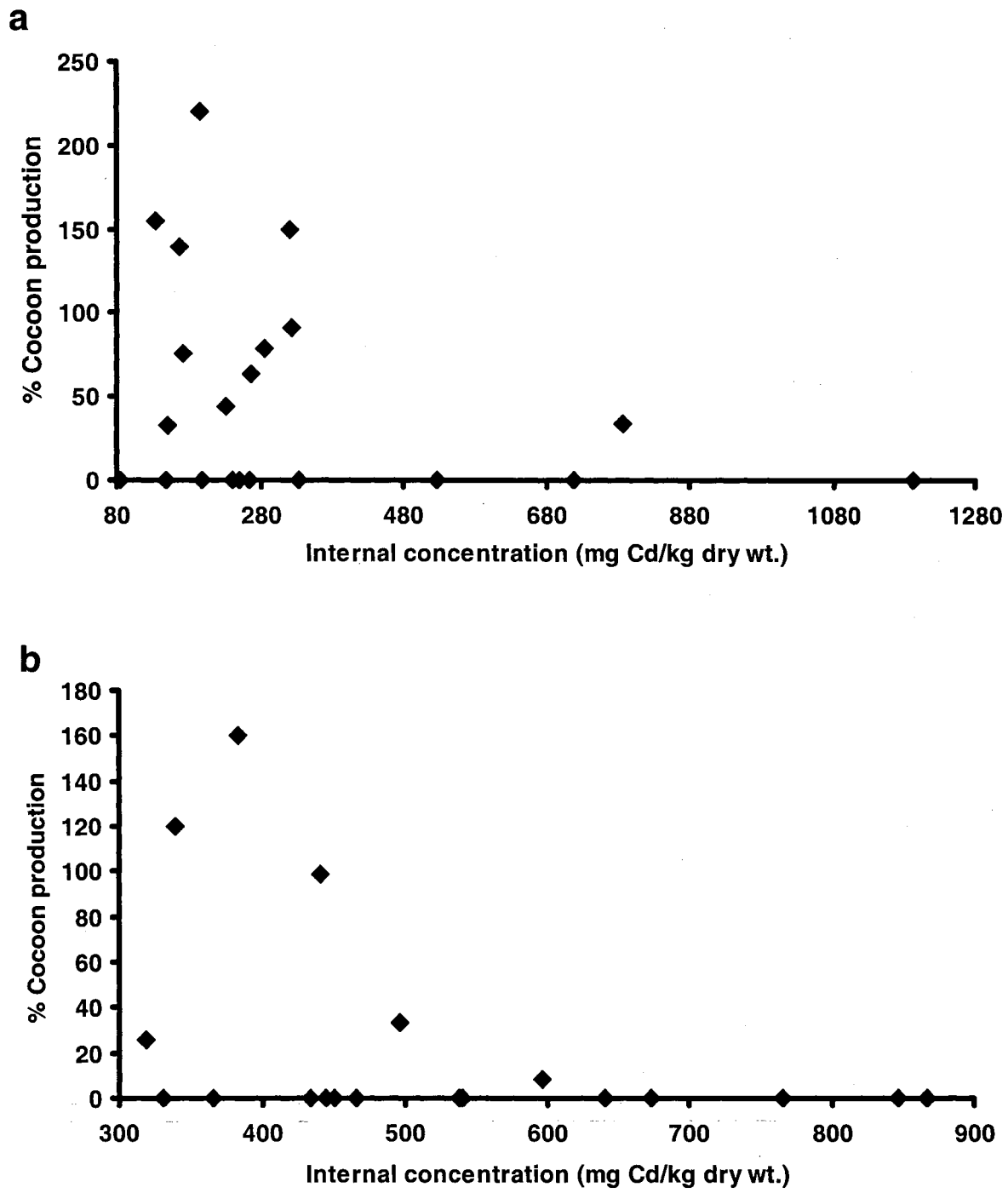

Figure 10. Cocoon production after 28-day exposure to (a) 50 mg Cd/kg and (b) 300 mg Cd/kg, expressed in percentage of control, versus internal concentrations.

Table 15. Biota-Soil Accumulation Factors for *Eisenia andrei* after 28-Day exposure to in 22 field soils spiked with Cd.

Soil	Horizon	Cd 50 mg/kg $BSAF_{Total}$[a]	Cd 50 mg/kg $BSAF_{Ca(NO3)2}$[b]	Cd 300 mg/kg $BSAF_{Total}$[a]	Cd 300 mg/kg $BSAF_{Ca(NO3)2}$[b]
		kg_s kg dry $weight_w^{-1}$	kg_s kg dry $weight_w^{-1}$	kg_s kg dry $weight_w^{-1}$	kg_s kg dry $weight_w^{-1}$
Bernow	B	12.6	11.6	2.01	2.49
Canisteo	A	4.14	24.1	1.44	10.1
Dennis	A	6.63	8.83	1.91	2.54
Dennis	B	5.41	9.18	1.43	2.28
Dougherty	A	7.05	8.82	3.02	3.59
Hanlon	A	4.13	13.1	1.59	3.66
Kirkland	A	5.89	7.59	1.77	2.12
Luton	A	2.67	10.7	1.04	2.88
Mansic	A	3.32	23.3	1.10	5.83
Mansic	B	1.81	17.2	1.07	12.6
Norge	A	3.34	4.49	0.27	0.33
Osage	A	3.38	8.48	2.43	5.17
Osage	B	4.05	8.39	1.25	2.27
Pond Creek	A	5.77	8.03	2.36	3.37
Pond Creek	B	15.4	16.4	2.83	3.39
Pratt	A	14.6	19.5	2.74	3.84
Pratt	B	29.7	24.2	1.98	1.83
Richfield	B	5.48	12.3	1.56	3.11
Summit	A	5.33	32.3	1.58	6.64
Summit	B	4.30	11.1	1.44	2.88
Taloka	A	6.26	7.85	2.43	3.16
Teller	A	1.48	2.11	1.24	1.59
MINIMUM		1.48	2.11	0.27	0.33
MAXIMUM		29.7	32.3	3.02	12.6
MEAN		6.94	13.2	1.75	3.89
MEDIAN		5.37	10.9	1.59	3.13

[a] $BSAF_{Total}$ calculated as mg Cd kg dry weight $worm^{-1}$:Total mg Cd kg dry weight $soil^{-1}$.
[b] $BSAF_{Ca(NO3)2}$ calculated as mg Cd kg dry weight $worm^{-1}$:$Ca(NO_3)_2$-extractable mg Cd kg dry weight $soil^{-1}$.

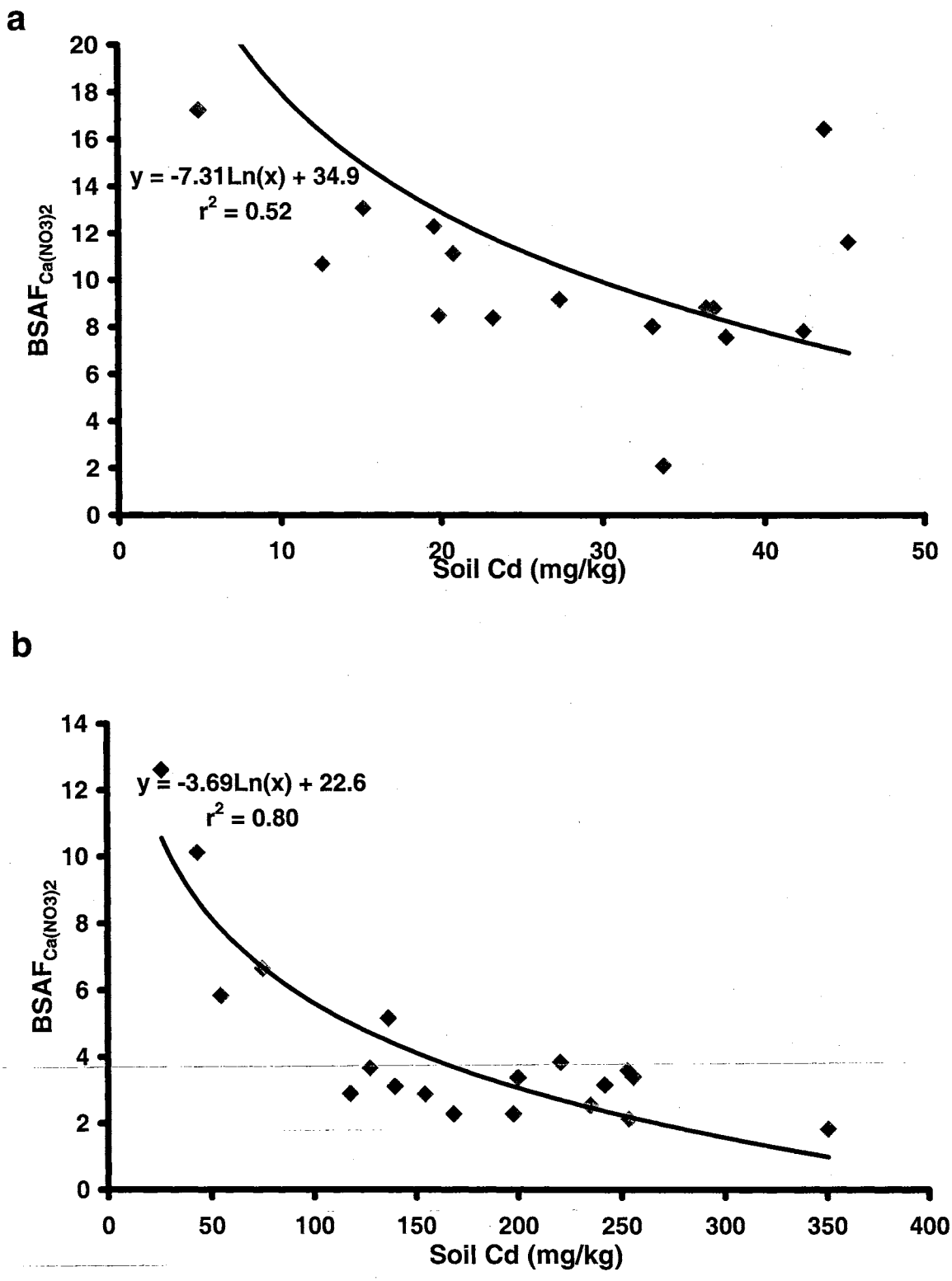

Figure 11. Biota-soil accumulation factors (BSAFs) as a function of $Ca(NO_3)_2$-extractable soil concentration of 50 mg Cd/kg (a) and 300 mg Cd/kg (b).

CHAPTER 3

EFFECT OF SOIL PROPERTIES ON THE BIOAVAILABILITY AND TOXICITY OF ZINC TO *EISENIA ANDREI*

ABSTRACT

Zn bioavailability and toxicity is directly influenced by soil properties. In the present study, the relationship between soil properties, and Zn bioavailability and toxicity in earthworms (*Eisenia andrei*) exposed to 22 field soils spiked with Zn is investigated to develop a mathematical model to describe this relationship. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to the same total Zn concentration in spiked soils. Statistical models were developed for earthworm mortality, cocoon production, internal concentrations, extractable Zn levels, and bioaccumulation factors. Internal Zn concentrations of earthworms were regulated to a constant level of approximately 136 mg Zn kg^{-1}. Results established a non-significant relationship for 0.5 M $Ca(NO_3)_2$-extractable Zn and internal Zn concentrations (P = 0.25). Bioaccumulation factors decreased with increasing available Zn concentrations indicating that bioaccumulation factors proved unreliable for assessing the influence of soil properties on the bioavailability of Zn. In soils with the largest bioaccumulation factors, little or no mortality was observed suggesting that bioaccumulation factors are poor indicators of Zn toxicity to earthworms. A significant relationship was established for mortality and internal Zn

concentrations suggesting that internal concentrations may be better suited as indicators of Zn toxicity ($P < 0.01$)

INTRODUCTION

Zinc (Zn) is an essential element in human and animal nutrition. However, excess amounts of Zn contamination of soil is a worldwide problem that may pose a risk to soil organisms. Sources of Zn contamination of soil include mining and smelter operations, industrial discharge, Zn fertilizer, and sewage sludge (Adriano, 2001). In order to protect and/or restore soil ecosystems, it is necessary to accurately characterize the risk posed to soil organisms by Zn and other contaminants. Risk is directly related to Zn bioavailability, which depends upon Zn concentration, the geochemical forms of Zn, the species of organism exposed, physical and chemical characteristics of the exposure environment, and the exposure duration. Bioavailability and toxicity are not permanent properties of soil but vary with the interaction between the soil and the organism (Lanno and McCarty, 1997). There are direct and indirect methods for determining bioavailability. Direct measures of bioavailability incorporate organismal responses and/or internal chemical concentrations to estimate bioavailability. Indirect measurements of bioavailability do not use living organisms to estimate the bioavailability of chemicals from soil. Instead, they utilize measured concentrations of chemical species that are potentially available for uptake. Weakly bound or available metals are believed to be available for uptake by earthworms (Posthuma et al., 1997; Peijnenburg et al., 1999b). The authors

suggested field soils should be tested to further validate the use of weak electrolyte extractions as surrogate measures of bioavailability. Only an organism can determine bioavailability or toxicity (Lanno and McCarty, 1997). However, it is necessary to integrate chemical (indirect) and biological (direct) measures to accurately reflect the bioavailability of Zn in soils and to protect ecological receptors.

Due to soil modifying factors, total Zn concentrations are inaccurate for predicting soil organism toxicity (Ma, 1982; Beyer at al., 1987; Morgan and Morgan, 1988; McLean and Bledsoe, 1992). Risk to soil organisms based on total contaminant concentration is not an accurate predictor of adverse effects (Peijnenburg et al., 1999a) because exposure expressed as total Zn does not consider the effects of soil modifying factors on Zn bioavailability. As an example, soil pH is inversely related to Zn solubility and availability (Adriano, 2001; Rieuwerts et al., 1998). Due to modifying factors, soil metal is often less than 100% available for uptake by organisms (Conder and Lanno, 2000). The objective of this study was to examine the effect of soil properties (e.g., pH, organic matter content, clay content), on Zn bioavailability and toxicity in earthworms (*Eisenia andrei*) and develop a mathematical model to describe this relationship.

METHODS AND MATERIALS

Soil collection and preparation were conducted using standard soil testing methods (See Schroder et al., in preparation, for complete methods). Twenty-

two soils with diverse paleoclimatology and geology were collected from Oklahoma and central Iowa to obtain Mollisols with a high organic C content. Soils were spiked with reagent grade $Zn(NO_3)_2$ to obtain soil concentrations of approximately 300 mg Zn kg^{-1}. Spiked soils were subjected to four wet-dry cycles (see Schroder et al., in preparation, for more detail) to achieve adequate reaction with the soil matrix and reduce the "salt effect" where heavy metal availability is greater in spiked soil than aged contaminated soil with similar metal contamination (Logan and Chaney, 1983). Total zinc in collected soils was determined by an acid digestion microwave technique according to U.S. EPA Method 3051 for confirmation of background Zn concentrations prior to analysis of chemical and physical properties (U.S. EPA 1994). Soil pH was determined in 1:1 soil:water suspension (Thomas, 1996). Soil organic C content was determined by acid dichromate digestion according to Heanes (1984). Cation exchange capacity of non-calcareous soil (soil pH < 7.0) was determined using a procedure adopted from Hendershot and Duquette (1986). Cation exchange capacity of calcareous soils (soil pH > 7.0) was determined according to the method of Polemio and Rhoades (1977). Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). $Ca(NO_3)_2$-extractable Zn was determined by placing soil (1.0 g) in a 50 mL centrifuge tube, extracting with 20.0 mL of 0.5 *M* $Ca(NO_3)_2$ solution, and shaking tubes on a reciprocal shaker for 16 h. The solutions were then centrifuged at 10,000 rpm for 15 min, filtered through a 0.45 μm membrane filter, acidified with 1.0 mL of trace metal concentrated HCl, and stored at 4 °C until analysis of metal by ICP-AES. Spiked soils were

digested by microwave according to U.S. EPA Method 3051 to determine total Zn concentrations. Blanks, spikes and certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were digested and analyzed for quality assurance and quality control in the determination of metal content in soil.

Earthworm bioassays

Twenty-eight day bioassays using *Eisenia andrei* were conducted with field soils spiked with 300 mg Zn kg^{-1}. The bioassays were performed in triplicate for each soil-Zn combination and conducted using mature (clitellate) manure worms (*E. andrei*) according to a standard protocol (American Society for Testing and Materials, 1997). The 200-g soil samples were moistened and maintained between 1/3 bar and saturation, placed in glass jars with 3 small air holes in the lid, and acclimated in an environmental chamber maintained at 20±1°C for 24 h prior to the addition of 10 earthworms per replicate. Twenty-four hours prior to the addition of earthworms to test soils, mature (clitellate) earthworms weighing approximately 0.2-0.4 g were removed from synchronized in-house cultures, rinsed with reagent grade water, and placed on moist filter paper for 24 hours to depurate most of the bedding material from their intestinal tracts (Van Gestel et al. 1993). At the start of the toxicity test, randomly chosen earthworms were removed from the filter paper, rinsed, and separated into replicates of 10 earthworms. Each replicate was blotted dry, weighed, and transferred to one of three jars prepared for each soil. Testing was conducted in an environmental chamber maintained at 20 ± 1°C with constant light. Earthworms were monitored

after six hours for physical condition and to determine if burrowing had occurred. Earthworms were observed daily for the first eight days and three times a week thereafter for the remainder of the test to assess the general condition of the worms and remove mortalities. Cocoons were collected on a daily basis by hand sorting. Simultaneously, observations on earthworm performance in ASTM artificial soil (American Society for Testing and Materials, 1997) and unspiked reference soils from each site served as controls for quality assurance with respect to survival, cocoon production, and growth. Artificial soils consisted of 69.5% silica sand, 20% kaolin clay, 10% 2-mm sieved *Sphagnum* peat moss, and approximately 0.5% $CaCO_3$ added to adjust the pH to 7.0±0.5. Reagent grade water was added to hydrate the artificial soil to 45% of its dry weight. All soil materials used were hydrated and allowed to acclimate in the environmental chamber maintained at 20±1°C 24 hours prior to the start of the tests. Earthworms were judged dead if no response was observed after gentle stimulation with a blunt probe. Dead earthworms were removed, rinsed thoroughly with reagent grade water, individually wrapped in aluminum foil, and frozen at -20°C for subsequent analysis. At day 28 of each study, live earthworms were depurated for 24 hours on moist filter paper, rinsed, weighed, and stored as described above. Upon the completion of toxicity tests, individual soil replicates from all experiments were stored at -20°C in Ziploc® freezer bags.

Internal concentrations

Earthworm Zn concentrations were determined as described by Morgan et al. (1982). An individual worm from each replicate (3 replicates per soil-Zn combination) was removed from the freezer, dried for 24 hours at 105°C in a pre-weighed 10 mL glass beaker, and weighed. Individual worms were then wet digested using 5 mL concentrated trace metal grade HNO_3 (Fisher Scientific). Digests were evaporated to dryness, resolubilized in 3 mL 0.5 M HNO_3, heated for 15 minutes at 60°C, and diluted to final volume of 10 mL with 0.5 M HNO_3. Worm digests were stored in Nalgene® low-density polyethylene bottles until analysis. Zn concentrations in digests were measured using flame AAS (PerkinElmer Analyst 700). The limit of detection for Zn in earthworm tissue digests was 2.5 μg/L. Zn concentrations in worm tissues were expressed on an mg kg^{-1} dry weight basis. All analyses included procedural blanks, spikes, and certified reference material (lobster hepatopancreas, TORT-2, National Research Council, Canada). Mean (%RSD) spike and certified reference material recoveries were 98 (4.1%) and 102 (3.2%), respectively.

Data analysis

Statistical analyses were performed using PC SAS Version 8.2 (SAS Institute Inc., Cary, NC). LC_{50} values were based on models produced by Proc Probit. Empirical models were developed for comparison to models in the literature. Backwards-stepwise regression analysis was used to derive empirical models capable of predicting effects of Zn on earthworm mortality, internal

concentrations, and cocoon production based on soil properties. The backwards-stepwise regression analysis was used to identify critical soil properties explaining most of the variation. Soil properties that did not explain a significant part of the variation ($P > 0.10$) were not used as independent variables in the multiple regression equation. Statistical models capable of predicting effects of Zn bioavailability, based on soil properties were obtained from the regression analysis. The multiple regression functions have the format:

$$Y = b_0 + b_1(pH) + b_2(clay) + b_3(OC)$$

Where Y = extractable Zn, earthworm response (mortality, internal concentrations, and cocoon production), or biota-soil accumulation factors, A, B, and C = soil properties (pH, clay, OC), and a, b, and c = coefficients.

Empirical models were compared with quantitative causal values for each soil property provided by path analysis models. Path analysis, an extension of the regression model, is a statistical technique that differentiates between correlation and causation (Basta et al., 1993). Path analysis was used to decompose correlations in the model into direct or causal effects of soil properties (Loehlin, 1987) on earthworm mortality, internal concentrations, and cocoon production. Path analysis allows the partitioning of simple correlation coefficients between dependent (e.g. mortality) and independent variables (soil properties) into direct and indirect effects (Basta et al., 1993). Path analysis also provides a numerical value for each direct and indirect effect and indicates the relative strength of that correlation or causal influence (Loehlin, 1987). Direct effects are standardized

partial regression coefficients designated as path coefficients (Basta et al., 1993). Direct and indirect effects are derived from multiple linear regression of soil properties on earthworm response (mortality, internal concentrations, and cocoon production) and simple correlation values between soil properties. In addition, an uncorrelated residual (U) was determined from this model using the following equation:

$$U = \sqrt{1 - R^2}$$

A path analysis model was composed to study the effect of pH, OC, and clay on earthworm mortality (Figure 1). Direct effects (path coefficients) of soil properties on earthworm mortality are represented by the single-headed arrows while the double-headed arrows represent intercorrelation coefficients. Indirect effects of soil properties on earthworm mortality are determined from the product of one double-headed arrow and one single-headed arrow (Basta et al., 1993). Path analysis results were derived using the following equations (Williams et al., 1990):

$$r_{14} = P_{14} + r_{12}P_{24} + r_{13}P_{34} \qquad [1]$$

$$r_{24} = r_{12}P_{14} + P_{24} + r_{23}P_{34} \qquad [2]$$

$$r_{34} = r_{13}P_{14} + r_{23}P_{24} + P_{34} \qquad [3]$$

where r_{i4} corresponds to the simple correlation coefficient between the soil property and earthworm response, P_{i4} are path coefficients (direct effects) of soil property i on earthworm response, and $r_{ij}P_{i4}$ are the indirect effects of soil property j through i on earthworm response. Subscript designations are: (1) pH, (2) OC, (3) Clay, and (4) earthworm response.

The path analysis results can be presented in a concise table (Williams et al., 1990). This table provides underlined diagonal numbers indicating direct effects and off-diagonal numbers indicating indirect effects. The position of each response in the table corresponds to its position in the matrix of respective equations (equations [1], [2], [3], above). This format allows all potential tables to be presented as one table.

RESULTS AND DISCUSSION

Metal availability

The 22 soils collected had a wide range of soil properties including soil pH (4.0–8.0), cation exchange capacity (3.0 to 32.4 $cmol_c\ kg^{-1}$), organic C (0.3 to 3.0%), and clay content (5.0 to 71%) (Table 16). The Zn content of collected soils was similar to uncontaminated background soil contents prior to Zn amendment (See Schroder et al., in preparation). The target value for Zn amended in the test soils was 300 mg Zn kg^{-1}, based upon earthworm responses in range-finder tests. All test soils were within 10% of the mean Zn content of 352 mg kg^{-1} (Table 17). The total Zn concentration of test soils ranged from 292 to 445 mg kg^{-1}. The mean $Ca(NO_3)_2$-extractable Zn concentration was 72.9 mg kg^{-1} and ranged from 0.8–60% of total Zn levels (Table 17). Conder and Lanno (2000) found that $Ca(NO_3)_2$-extractable Zn levels in artificial soils were 0-72% of total Zn levels. The 75-fold difference found for $Ca(NO_3)_2$-extractable Zn concentrations

expressed as a percent of total Zn in this study suggests that soil properties profoundly modulated extractable Zn levels.

Extraction techniques using weak salt solutions (< 1*M* $CaCl_2$ or $Ca(NO_3)_2$) estimate metal levels in soil pore water and readily dissolved metal adsorbed to soil components or in minerals with high water solubility. These forms of extractable metal are a more accurate measure of actual metal exposure than total metal levels (Lanno et al., 1999). This type of extraction technique has shown promise as a toxicity-related measure of bioavailability in soils (Conder and Lanno, 2000; Basta and Gradwohl, 2000; Posthuma et al., 1997; Sloan et al., 1997; Peijnenburg et al., 1997, 1999b; Weljte, 1998).

Because surrogate measures of bioavailability must be correlated with organismal responses (Lanno et al., 2002, in press), models were developed using both direct (internal concentrations) and indirect approaches (organismal responses and weak electrolyte extractions) for assessing Zn bioavailability and toxicity. Models were developed for earthworm mortality, cocoon production, internal concentrations, extractable Zn, and bioaccumulation factors. Path analysis results for soils tested are listed in Table 18. Simple correlation coefficient (*r*) values between pH, OC, clay, and extractable Zn concentrations are listed for comparison with path analysis results. Path analysis partitions each *r*-value into one direct effect (underlined, main diagonal positions) and two indirect effects (off diagonal positions). Significant direct effects are indicated by

corresponding *p*-values for each model tested. Significant coefficient of determination (R^2) values and low uncorrelated (U) values indicate that the path analysis model explains most of the variation in each of the models tested. The model explains most of the variation observed in $Ca(NO_3)_2$-extractable Zn (R^2 = 0.59, $P < 0.01$) (Table 18). Simple correlation results (r-values) indicate that that soil pH and clay strongly affected $Ca(NO_3)_2$-extractable Zn ($P < 0.01$). However, clay did not retain significance when partitioned into direct and indirect effects by path analysis. Simple correlation coefficients are significant due to indirect effects or intercorrelations of soil properties. Path analysis partitioning provides direct effects or causation of soil properties on $Ca(NO_3)_2$-extractable Zn. Path analysis direct effects show that soil pH affected $Ca(NO_3)_2$-extractable Zn ($P < 0.05$). Spurgeon and Hopkin (1996) showed that the concentration of soluble Zn was greatest in artificial soils with low pH and OM contents.

Regression analysis results for the 22 Zn spiked soils tested are listed in Table 19. Backwards-stepwise regression was used to identify the critical soil properties that explain most of the variation of these parameters in 22 field soils. Backwards stepwise regression results indicate that clay and pH explained the variance among $Ca(NO_3)_2$-extractable Zn concentrations (R^2 = 0.58, $P < 0.01$) and were predictive of available Zn in soil. Previous research has indicated that pH is typically the main factor modulating metal solubility and bioavailability (McLean and Bledsoe, 1992; Peijnenburg et al., 1997, 1999b; Posthuma et al., 1997; Basta et al., 1993; Smit et al., 1998).

Earthworm mortality

Cumulative mean (n = 66) earthworm mortality was 1.2%, which was < 10% in each of the 22 unspiked reference soils. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to Zn spiked soils (Figure 13). Adverse physiological responses to Zn exposure included yellow secretions, typical of stress responses in *E. andrei* (Edwards and Bohlen, 1992). In soils with 100% mortality (Norge A and Teller A), earthworms died during the first week of the experiment. Soils with low pH resulted in many dead animals within the first day. This was the case for Norge A and Teller A soils which had 100% mortality by day 2. According to Spurgeon et al. (1994), this may suggest that the main toxic effect was exerted by uptake across the body wall, rather than via dietary metal assimilation. Similar results were reported for *Eisenia fetida* exposed to 2000 μg Zn g^{-1} spiked artificial soil (Spurgeon et al., 1994). The range of mortality observed in our study was the result of differences in Zn bioavailability due to Zn interactions with the soil properties, assuming similar behavior of earthworms in each soil.

Results indicate that the model did not adequately explain the variability in mortality (R^2 = 0.30) and could not be used with confidence to explain the variability in mortality (Table 18). In part, this finding may be attributed to the high percentage of survival observed in these soils. Simple correlation results (*r*-values) indicate that soil pH significantly affected mortality ($P < 0.05$). However,

pH did not retain significance when partitioned into direct and indirect effects by path analysis.

Backwards-stepwise regression results reveal that pH and OC were more important than clay for modifying Zn bioavailability for mortality ($R^2 = 0.55$, $P < 0.01$) (Table 19). Spurgeon and Hopkin (1996) observed that Zn toxicity for *Eisenia fetida* decreased with an increase in pH and OC content of artificial soil. Soils containing higher organic matter content have a higher binding capacity for Zn (Adriano, 2001), potentially causing Zn to be unavailable for uptake by organisms.

Internal concentrations

The mean (SD) internal concentration of *E. andrei* exposed to unspiked reference soils was 147 mg Zn kg^{-1} (25.9) (Table 20). This is consistent with previous findings of approximately 100 mg Zn kg^{-1} in *Eisenia fetida* exposed to uncontaminated artificial soil (Spurgeon and Hopkin, 1996). The mean (SD) Zn concentration of earthworms exposed to 300 mg Zn kg^{-1} was 136 mg Zn kg^{-1} (34.1) (Table 20). Earthworms exposed to the Norge A and Teller A soils contained only 64.9 and 38.2 mg Zn kg^{-1}. Excluding the Norge A and Teller A earthworms, in which 100% mortality was observed by day 2, the internal Zn concentration was regulated to approximately 145 mg Zn kg^{-1}. Results are consistent with previous findings of internal Zn regulation by earthworms (Van Gestel, et al., 1993; Lock, 2001; Heikens, 2001).

Earthworms exposed to soils where 100% mortality occurred were removed from internal Zn concentration statistical models to avoid differences in Zn uptake due the physiological effects of acutely toxic Zn exposure. Results indicate that the model did not adequately explain the variability in internal concentrations (R^2 = 0.33) and could not be used with confidence to explain the variability in internal concentrations (Table 18). In part, this finding may be attributed to the regulation of internal Zn concentrations to a constant level of approximately 128 to 188 mg Zn kg^{-1}. The low R^2 and high U values may suggest that factors other than soil pH, OC, and clay are needed to explain this model. Simple correlation results (*r*-values) indicate that soil clay significantly affected internal concentrations (P < 0.05). However, clay did not retain significance when partitioned into direct and indirect effects by path analysis. Backwards-stepwise regression results indicate that clay contributed more than pH or OC to explaining the variance among internal concentrations (R^2 = 0.26, P = 0.02) (Table 19). This, however, conflicts with data given by Ma (1982) indicating that soil pH was the primary factor influencing Zn uptake. Regression analysis established a non-significant relationship for 0.5 M $Ca(NO_3)_2$-extractable Zn and internal Zn concentrations (P = 0.25).

Earthworm detoxification mechanisms for Zn are not as well studied as that of Cd. Earthworm chloragosomes may be capable of sequestering Zn when excess amounts due to contamination are present and releasing Zn to meet

physiological needs (Morgan and Morgan, 1989). Research suggests chloragosomes possess cation exchange capacities and an exchange mechanism for accumulation of chloragosomal Zn, thereby causing reduced chloragosomal Ca (Morgan and Morgan, 1989). High excretion rates for Zn indicate that it is further detoxified by excretion (Spurgeon and Hopkin, 1999). Excess Zn elimination by earthworms is most likely dependent on the essential nature of the element (Hopkin, 1995). Therefore, physiological pathways exist for the control of Zn (Spurgeon and Hopkin, 1999). Adverse effects can be expected only when the capacity of detoxification mechanisms is exceeded, occurring at a very high internal concentration when metal is slowly sequestered (Lock, 2001). Resulting mortalities in Norge A and Teller A suggests that detoxification and/or elimination mechanisms were exceeded. A significant relationship was found for internal concentrations and mortality ($r^2 = 0.65$, $P < 0.01$).

Cocoon production

Figure 15 illustrates a poor relationship for cocoon production of *E. andrei* and internal concentrations due to internal Zn concentrations being regulated within a narrow range. This finding is consistent with data for *Eisenia fetida* when exposed to Zn spiked artificial soils (Lock, 2001; Spurgeon and Hopkin, 1996). The model accounts for the variation observed in cocoon production ($R^2 = 0.42$, $P < 0.05$) (Table 18). Simple correlation results reveal that OC influenced cocoon production ($P < 0.01$). Path analysis partitioning indicated a strong causative or

direct effect of OC on cocoon production ($P < 0.01$). The natural habitats for *E. andrei* are those of very high organic matter such as manure or compost piles (ASTM, 1997), which may result in the direct effect of OC on cocoon production. However, OC was not removed from statistical models because this may result in inaccurate conclusions of significant effects on cocoon production. Backwards-stepwise regression indicates OC, along with a pH-OC interaction, contribute more than pH or clay alone to explaining the variance among cocoon production ($R^2 = 0.46$, $P < 0.01$) (Table 19).

Biota-soil accumulation factors

Biota-soil accumulation factors were developed for this study based on total Zn concentrations ($BSAF_{Total}$) and weak electrolyte extractions ($BSAF_{Ca(NO3)2}$). Biota-soil accumulation factors represent the ratio of internal Zn concentrations in exposed earthworms to concentrations in the exposure matrix. Calculated $BSAF_{Total}$ and $BSAF_{Ca(NO3)2}$ values are listed in Table 21. Biota-soil accumulation factors ranged approximately 4-fold from 0.13 – 0.55 for $BSAF_{Total}$ and over two orders of magnitude from 0.21 – 52.0 for $BSAF_{Ca(NO3)2}$. Results based on total soil Zn are consistent with previous findings (Posthuma, 1998). Because BSAFs are assumed to be independent of soil Pb concentrations, they are often used to assess the effect of soil properties on bioavailability (Peijnenburg et al., 1999a, 1999b; Janssen, 1997). Results show $BSAF_{Ca(NO3)2}$ values decreased according to a power function with increasing extractable Zn concentrations (Figure 16).

Significant coefficient of determination (R^2) and low uncorrelated (U) values indicate that the model explains the variation in the $BSAF_{Ca(NO3)2}$ model (Table 18). Simple correlation results indicated a significance of pH on $BSAF_{Ca(NO3)2}$ ($P < 0.01$). Path analysis partitioning for $BSAF_{Ca(NO3)2}$ indicates that pH remained a significant causative or direct effect on $BSAF_{Ca(NO3)2}$ ($P < 0.01$). Backwards-stepwise regression results suggest that OC, in addition to pH and a pH-OC interaction, is significant in explaining the variance among $BSAF_{Ca(NO3)2}$ ($R^2 = 0.68$, $P < 0.01$) (Table 19). However, path analysis models generated indicate that clay is significant for bioaccumulation factors based on total Zn and pH is significant for bioaccumulation factors based on $Ca(NO_3)_2$-extractable Zn.

Bioaccumulation factors are frequently used to assess the effect of soil-modifying factors on the bioavailability of metals in soils (Janssen et al., 1997; Peijnenburg et al., 1999a, 1999b). However, there is much debate about the use of bioaccumulation factors to assess the bioavailability of metals. Some authors report that bioaccumulation factors should be questioned because they tend to decrease with increasing metal concentrations, indicating there is no relationship between the internal concentration and the bioavailable concentration (Lock, 2000). Others state that bioaccumulation factors are more appropriate than body concentrations for normalization among field soils (Janssen, 1997). Bioaccumulation factors are usually normalized to total metal concentrations in soil. Peijneburg et al. (1999), suggests bioaccumulation factors should be based on bioavailable concentrations in the soil. In this study, bioaccumulation factors based on $Ca(NO_3)_2$-extractable Zn were found to decrease with increasing

concentrations of available Zn. It is assumed that bioaccumulation occurs when BSAFs are greater than one (Lock, 2001). Bioaccumulation factors based on total Zn in this study were consistently less than unity in all soils. However, bioaccumulation factors based on available concentrations in this study found many of the values were greater than one. In soils with the largest $BSAF_{Ca(NO3)2}$ values, little or no mortality was observed suggesting that $BSAF_{Ca(NO3)2}$ are poor indicators of adverse effects of Zn to earthworms ($P = 0.52$).

Empirical formulas developed may be useful for predicting the potential environmental risks of Zn in soil. Overall, pH was the most important soil property modifying the bioavailability and toxicity of Zn. Conclusions of our study do not support the use of weak-electrolyte extractions as a surrogate measure of Zn bioavailability. The significant relationship found for internal concentrations and mortality suggests that internal concentrations may prove useful as indicators of adverse effects of Zn toxicity and bioavailability to earthworms. Biota-soil accumulation factors in this study were deemed as poor indicators of environmental risk of Zn toxicity. Furthermore, the decrease in $BSAF_{Ca(NO3)2}$ with increasing available Zn concentrations indicates that BSAFs should not be used assess the influence of soil properties on Zn bioavailability.

Acknowledgements

We would like to thank Derrick Chappell for assistance with bioassays. The research in this manuscript has been funded by the U.S. Environmental

Protection Agency, Office of Research and Development, National Center for Exposure Assessment. Although the research described in this paper has been funded by the U.S. EPA to Dr. Roman Lanno and Dr. Nicholas Basta, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

Adriano, DC. 2001. *Trace elements in terrestrial environments.* Springer, New York, USA.

American Society for Testing and Materials. 1997. Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with Lumbricid earthworm *Eisenia fetida*, standard E 1676-97. In: Annual Book of ASTM Standards, pp. 1056-1074.

Basta NT, Pantone DJ, Tabatabai MA. 1993. Path analysis of heavy metal adsorption by soil. *Agron. J.* 85:1054-1057.

Basta NT, Gradwohl R. 2000. Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soil by a sequential extraction procedure. *Journal of Soil Contamination.* 9:149-164.

Beyer WN, Hensler G, Moore, J. 1987. Relation of pH and other soil variables to concentrations of Pb, Cu, Zn, Cd, and Se in earthworms. *Pedobiologia.* 30:167-172.

Conder JM, Lanno RP. 2000. Evaluation of cadmium, lead, and zinc to *Eisenia fetida*. *Chemosphere*. 41:1659-1668.

Edwards CA, Bohlen PJ. 1992. The effects of toxic chemicals on earthworms. *Rev. Environ. Contam. Toxicol.* 125:23-99.

Gee GW, Bauder JW. 1986. Particle-size analysis. In A. Klute, ed., *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods,* 2nd ed. Agronomy Monograph 9. Soil Science Society of America, Madison, WI, USA, pp. 383-411.

Heanes DL. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Commun. In Soil Sci. Plant Analysis.* 15:1191-1213.

Heikens A, Peijnenburg WJGM, Hendriks AJ. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. *Environ. Poll.* 113:385-393.

Hendershot WH, Duquette M. 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Sci. Soc. Am. J.* 50:605-608.

Janssen RPT, Posthuma L, Baerselman R, Den Hollander HA, Van Veen RPM, Peijnenburg WJGM. 1997. Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal accumulation in earthworms. *Environ. Toxicol. Chem.* 16:2479-2488.

Lanno R. Ed. 2002 (In press). *Contaminated soils: From soil-chemical interactions to ecosystem management. Proceedings of Workshop on Assessing Contaminated Soils, Pellston, MI, 23-27 September 1998.*

Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, USA.

Lanno RP, Conder JM, Seals L. 1999. Critical limits for heavy metals in soils and surface waters. *Workshop on effects-based approaches for heavy metals.* Federal Environmental Agency (Umweltbundesamt), Berlin, October.

Lanno RP, McCarty LS. 1997. Earthworm bioassays: Adopting techniques from aquatic toxicology. *Soil Biol. Biochem.* 29:693-697.

Lock K, Janssen CR. 2001. Zinc and cadmium body burdens in terrestrial Oligochaetes: use and significance in environmental risk assessment. *Environ. Toxicol. Chem.* 20:2067-2072.

Lock K, Janssen CR, De Coen WM. 2000. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to *Enchytraeus albidus*. *Environ. Toxicol. Chem.* 19:2666-2671.

Loehlin JC. 1987. *Latent variable models: an introduction to factor, path, and structural analysis.* Lawrence, Hillsdale, New Jersey, USA.

Ma WC. 1982. The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. *Pedobiologia* 24:109-119.

McLean LE, Bledsoe BE. 1992. Ground water issue: Behavior of metals in soils. EPA/540/S-92/018. U.S. Environmental Protection Agency, Washington, DC.

Morgan JE, Morgan AJ. 1989. Zinc sequestration by earthworm (Annelida: Oligochaeta) chloragocytes. *Histochem.* 90:405-411.

Morgan ME, Morgan AJ. 1988. Earthworms as biological monitors of Cd, Cu, Pb, and Zn in metalliferous soils. *Environ. Pollut.* 54:123-138.

Morgan AJ, Morris B. 1982. The accumulation and intracellular compartmentation of cadmium, lead, zinc, and calcium in two earthworm species (*Dendrobaena rubida* and *Lumbricus rubellus*) living in highly contaminated soil. *Histochem.* 75:269-285.

Peijnenburg WJGM, Posthuma L, Zweers PGPC, Baerselman R, de Groot AC, van Veen RPM, Jager T. 1999a. Prediction of metal bioavailability in Dutch field soils for the Oligochaeta *Enchytraeus crypticus*. *Ecotoxicol. Environ. Safety* 43:170-186.

Peijnenburg WJGM, Baerselman R, de Groot AC, Jager T, Posthuma L, Van Veen RPM. 1999b. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. *Ecotoxicol. Environ. Saf.* 44:294-310.

Peijnenburg WJGM, Posthuma L, Eijsackers HJP, Allen HE. 1997. A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicol. Environ. Safety* 37:163-172.

Polemio M, Rhoades JD. 1977. Determining cation exchange capacity: a new procedure for calcareous and gypsiferous soils. *Soil Sci. Soc. Am. J.* 41:524-528.

Posthuma L, Notenboom J, De Groot AC, Peijnenburg WJGM. 1998. Soil acidity as major determinant of zinc partitioning and zinc uptake in 2

Oligochaete worms exposed in contaminated field soils. In: Sheppard S, Bembridge J, Holmstrup M, Posthuma L. eds, *Advances in Earthworm Ecotoxicology: A Special Publication of* SETAC, CRC Press, Boca Raton, Florida, p. 111-127.

Posthuma L, Baerselman R, Van Veen RPM, Dirven-Van Breemen EM. 1997. Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. *Ecotoxicol. Environ. Safety* 38:108-121.

Rieuwerts JS, Thornton I, Farago ME, Ashmore, MR. 1998. Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chem. Spec. Bioavail.* 10:61-75.

Schroder J. 2002 (In preparation). Ph.D. Dissertation. Oklahoma State University, Stillwater, OK.

Sloan JJ, Dowdy RH, Dolan MS, Linden DR. 1997. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. *J. Environ. Qual.* 26:966-974.

Smit CE, Posthuma L, Folkerts AJ, Baerselman R, Dirven-Van Breemen EM, Bogte J, Van Beelen P. 1998. Heavy metal behavior in OECD artificial soil, Budel gradient soils and experimental field plot soil-methodological aspects and soil chemical characterization of contamination. In: Posthuma L, Van Gestel CAM, Smit CE, Bakker DJ, Vonk JW. eds, *Validation of Toxicity Data and Risk Limits for Soils: Final Report.*

National Institute of Public Health and Environment (RIVM), Bilthoven, The Netherlands, p. 230.

Spurgeon DJ, Hopkin SP. 1999. Tolerance to zinc in populations of the earthworm *Lumbricus rubellus* from uncontaminated and metal-contaminated ecosystems. *Arch. Environ. Contam. Toxicol.* 37:332-337.

Spurgeon DJ, Hopkin SP. 1996. Effects of variations of the organic matter content and pH of soils on the availability and toxicity of zinc to the earthworm *Eisenia fetida*. *Pedobiologia* 40:80-96.

Spurgeon DJ, Hopkin SP, Jones DT. 1994. Effects of Cd, Cu, Pb, and Zn on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ. Poll.* 84:123-130.

Thomas GW. 1996. Soil pH and soil acidity. In Sparks DL, ed, *Methods of Soil Analysis.* Part 3 Chemical Methods-SSSA Book series no. 5. Soil Science Society of America, Madison, WI, pp. 475-490.

U.S. Environmental Protection Agency. 1994. Method 3051: Microwave assisted acid digestion of sediment, sludges, soils, and oils.

Van Gestel CAM, Dirven-van Breeman EM, Baerselman R. 1993. Accumulation and elimination of cadmium, chromium, and zinc and the effects on growth and reproduction of *Eisenia andrei* (Oligochaeta, Annelida). *Sci. Total Environ. Supplement* 585-597.

Weljte L. 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb, and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. *Chemosphere* 36:2643-2660.

Williams WA, Jones MB, Demment MW. 1990. A concise table for path analysis statistics. *Agron. J.* 82:1022-1024.

Table 16. Properties of Soils

Soil	Horizon	Unspiked Soil pH[a]	Zn spiked soil pH[a]	CEC cmol/kg[b]	OC	Sand	Clay	Silt	Class
						%			
Bernow	B	4.9	3.9	6.74	0.30	58.8	26.3	17.5	Sandy Clay Loam
Canisteo	A	7.5	7.7	30.5	3.00	31.3	38.8	51.3	Silt Loam
Dennis	A	5.6	4.7	9.77	1.90	37.5	23.8	40.0	Loam
Dennis	B	6.1	5.3	14.6	0.80	21.3	45.0	40.6	Clay Loam
Doughtery	A	5.3	4.6	3.33	1.20	75.0	11.3	21.3	Loan Sand
Hanlon	A	7.4	6.4	16.3	1.60	63.8	17.5	23.8	Sandy Loam
Kirkland	A	5.6	4.8	14.0	1.45	12.5	31.3	57.5	Silty Clay Loam
Luton	A	7.1	6.8	32.4	2.00	2.50	71.3	38.8	Clay
Mansic	A	7.8	7.7	16.5	1.50	33.8	30.0	43.8	Loam
Mansic	B	8.0	7.2	11.7	0.53	30.0	35.0	42.5	Clay Loam
Norge	A	4.0	3.9	4.57	1.20	36.9	17.5	45.6	Silt Loam
Osage	A	6.6	5.8	28.3	2.60	13.8	55.7	53.8	Silty Clay Loam
Osage	B	6.8	6.2	27.5	2.00	11.3	61.3	47.5	Silty Clay
Pond Creek	A	5.2	5.8	10.7	1.90	16.3	28.8	62.5	Silt Loam
Pond Creek	B	6.0	5.4	12.5	0.80	18.8	32.5	48.8	Silty Clay Loam
Pratt	A	6.5	5.3	4.40	0.90	90.0	5.00	3.80	Silt
Pratt	B	6.4	4.4	3.40	0.50	92.5	6.25	1.30	Silt
Richfield	B	7.7	6.9	22.3	1.10	11.3	41.3	51.3	Silty Clay Loam
Summit	A	7.2	7.3	29.4	2.40	17.5	45.7	53.8	Silty Clay Loam
Summit	B	7.1	6.4	27.6	1.25	10.0	56.8	48.8	Silty Clay
Taloka	A	5.1	4.3	4.85	1.20	20.0	11.3	58.8	Silt Loam
Teller	A	4.5	4.1	3.01	0.85	66.9	10.0	23.8	Silt Loam
MINIMUM		4.0	3.9	3.01	0.30	2.50	5.00	1.30	
MAXIMUM		8.0	7.7	32.4	3.00	92.5	71.3	62.5	
MEAN		6.3	5.7	15.2	1.41	35.1	32.0	40.0	
MEDIAN		6.5	5.6	13.3	1.25	25.7	30.7	45.0	

[a] pH determined by 1:1 soil:water
[b] Cation Exchange Capacity measured using 0.1 M $BaCl_2$ for non-calcareous soils; 1 M NaOAc, pH 8.2 for calcareous soils.

Table 17. Zn soil concentrations.

Soil	Horizon	$Ca(NO_3)_2$-extractable Zn[a] mg kg^{-1}	%RSD	%[b]	Total Zn[c] mg kg^{-1}	%RSD
Bernow	B	112	1.86	33.7	334	0.42
Canisteo	A	3.85	6.01	1.28	300	4.66
Dennis	A	97.9	7.75	27.7	353	0.67
Dennis	B	17.7	2.77	4.75	373	2.56
Dougherty	A	171	3.34	54.6	312	1.43
Hanlon	A	45.1	7.68	13.1	344	3.01
Kirkland	A	143	1.64	42.0	340	0.81
Luton	A	7.61	18.6	1.72	442	0.40
Mansic	A	7.25	20.4	2.01	360	6.82
Mansic	B	2.59	9.50	0.83	314	1.03
Norge	A	163	6.74	55.0	296	0.17
Osage	A	48.4	1.24	11.0	441	2.10
Osage	B	34.7	0.51	7.81	445	0.08
Pond Creek	A	153	2.05	43.0	356	4.64
Pond Creek	B	61.7	4.56	18.5	333	0.09
Pratt	A	198	1.53	54.1	365	3.13
Pratt	B	102	9.90	35.0	292	2.65
Richfield	B	20.1	2.82	5.07	396	8.27
Summit	A	6.13	12.2	1.69	363	0.25
Summit	B	22.4	40.1	6.16	363	0.04
Taloka	A	5.95	8.28	1.90	314	1.64
Teller	A	182.2	3.85	60.1	303	0.37
MINIMUM		2.59	0.51	0.83	292	0.04
MAXIMUM		198	40.1	60.1	445	8.27
MEAN		72.9	7.88	21.9	352	2.06
MEDIAN		46.7	5.28	12.1	349	1.23

[a] Extracted using 0.5 M $Ca(NO_3)_2$, mean (n=3)
[b] Percent of total metal that was $Ca(NO_3)_2$-extractable
[c] Extracted according to EPA Method 3051 and measured by ICP-AES, mean (n=2)

Table 18. Path analysis direct effects (bold diagonal, underlined) and indirect effects (off diagonal) of soil pH, organic carbon (% OC), and clay (mmol kg^{-1}) on extractable Zn concentrations and *Eisenia andrei* after 28-day exposure to Zn.

Response		pH	OC	Clay	r	R^2	U
$Ca(NO_3)_2$	pH	**-0.56***	0.08	-0.23	-0.70**	0.59**	0.64
extractable Pb	OC	-0.28	**0.16**	-0.19	-0.30		
(mg/kg)	Clay	-0.32	0.08	**-0.39**	-0.63**		
% Mortality	pH	**-0.48**	0.07	-0.12	-0.52*	0.30	0.84
	OC	-0.24	**0.14**	-0.10	-0.20		
	Clay	-0.28	0.07	**-0.20**	-0.41		
Internal	pH	**0.19**	0.08	-0.36	-0.09	0.33	0.82
concentration[a]	OC	0.09	**0.17**	-0.32	-0.06		
(mg/kg dry wt.)	Clay	0.10	0.08	**-0.69***	-0.51*		
Cocoon	pH	**-0.30**	0.35	0.07	0.12	0.42*	0.76
Production[a,b]	OC	-0.15	**0.70****	0.06	0.61**		
	Clay	-0.18	0.34	**0.12**	0.28		
$BSAF_{Ca(NO3)2}$[b]	pH	**0.76****	-0.08	-0.08	0.60**	0.41*	0.77
	OC	0.37	**-0.16**	-0.07	0.14		
	Clay	0.40	-0.07	**-0.15**	0.17		

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Cumulative mean cocoons per soil-Zn combination (n=3)

[b] Soils with 100% mortality removed from model

Table 19. Multiple regression formulae describing the quantitative relationship between soil properties, *Eisenia andrei* after 28-day exposure to Zn, and extractable Zn concentrations.

Response	Regression equation obtained[a]	Statistics
$Ca(NO_3)_2$-extractable Zn (mg/kg)	$y = 194.8 - 1.288(clay) - 2.393(pH)^2$	$R^2 = 0.58$, n = 22, $P < 0.01$
% Mortality	$y = 436.8 - 156.4(pH) + 68.26(OC) + 12.42(pH)^2 - 20.10(OC)^2$	$R^2 = 0.55$, n = 22, $P < 0.01$
Internal concentration[b] (mg/kg dry wt.)	$y = 159.1 - 0.446(clay)$	$R^2 = 0.26$, n = 20, $P = 0.02$
Cocoon Production[b]	$y = -5.307 + 14.21(OC) - 1.189(pH*OC)$	$R^2 = 0.46$, n = 20, $P < 0.01$
$BSAF_{Ca(NO3)2}$[b]	$y = 113.2 - 44.11(pH) + 5.051(pH)^2 + 10.40(OC)^2 - 5.752(pH*OC)$	$R^2 = 0.68$, n = 20, $P < 0.01$

[a] All variables in the models are significant ($P < 0.1$)

[b] Soils with 100% mortality were removed from model

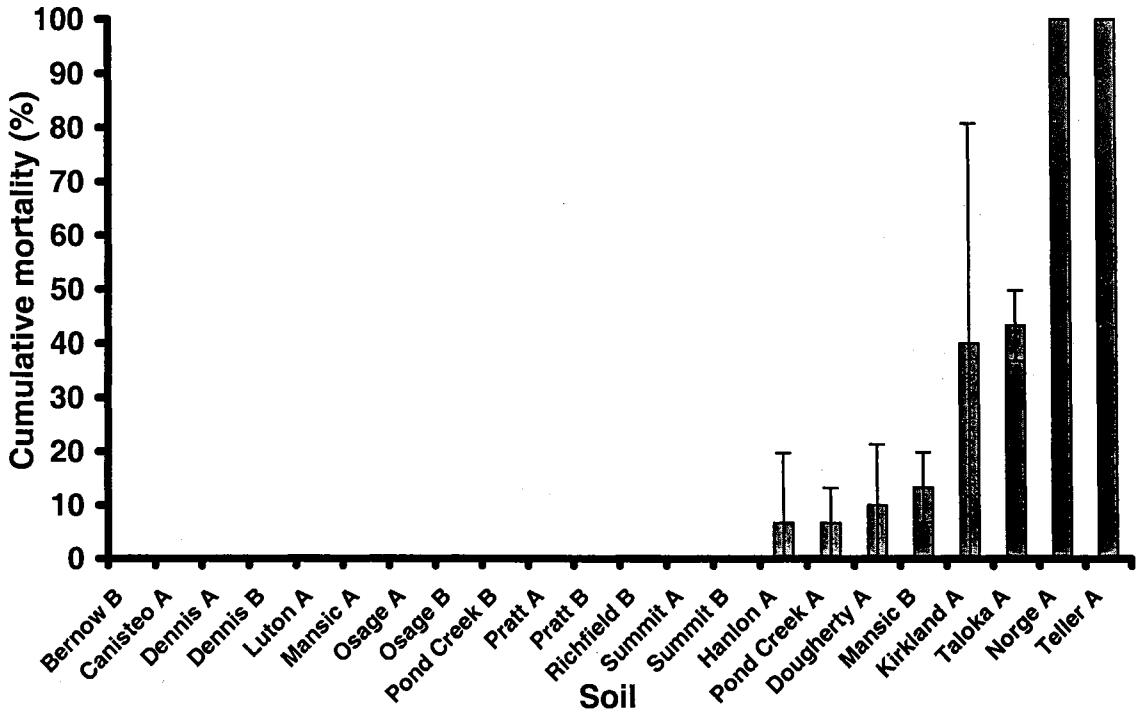

Figure 12. Cumulative mortality (mean, n =3 per soil-Zn combination, ± 95% CI) of *Eisenia andrei* exposed to 300 mg Zn/kg spiked soils for 28 days.

Table 20. Internal concentrations of Eisenia andrei exposed to unspiked and Zn spiked soils (mean, n = 3).

Soil	Horizon	Unspiked soil earthworm concentration[1] mg kg^{-1}	%RSD	Zn spiked soil earthworm concentration[2] mg kg^{-1}	%RSD
Bernow	B	139	4.89	136	4.20
Canisteo	A	141	2.12	129	0.49
Dennis	A	164	12.9	151	16.5
Dennis	B	140	9.74	142	7.05
Dougherty	A	160	4.86	150	7.24
Hanlon	A	149	13.1	155	15.9
Kirkland	A	152	2.41	137	11.7
Luton	A	162	9.24	128	20.6
Mansic	A	189	24.3	153	25.1
Mansic	B	141	13.7	135	20.9
Norge	A	105	12.8	64.9	21.8
Osage	A	183	32.5	139	15.2
Osage	B	156	5.48	139	2.77
Pond Creek	A	148	23.9	138	6.66
Pond Creek	B	123	0.24	135	17.3
Pratt	A	140	15.2	188	5.09
Pratt	B	122	8.09	161	13.2
Richfield	B	139	4.27	140	11.9
Summit	A	164	4.68	171	14.4
Summit	B	130	5.53	132	11.5
Taloka	A	162	4.63	143	8.39
Teller	A	132	41.3	38.2	20.0
MINIMUM		105	0.24	38.2	0.49
MAXIMUM		189	41.3	188	25.1
MEAN		147	11.6	136	12.6
MEDIAN		144	8.67	139	12.5

[1] Zn concentration in digests of worms exposed to reference (unspiked) soils, measured by FAAS (limit of detection 2.5 μg/L)

[2] Zn concentration in digests of worms exposed to 300 mg/kg Zn spiked soils, measured by FAAS (limit of detection 2.5 μg/L)

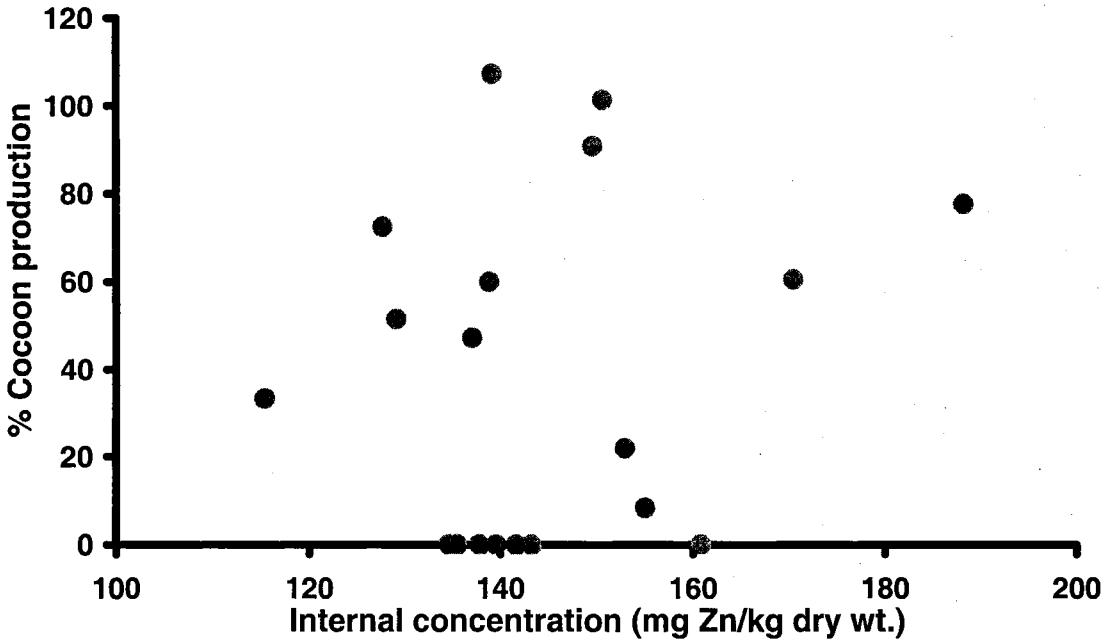

Figure 13. Cocoon production after 28-day exposure, expressed in percentage of control, versus internal concentrations.

Table 21. Biota-Soil Accumulation Factors for *Eisenia andrei* after 28-Day exposure to in 22 field soils spiked with Zn.

Soil	Horizon	$BSAF_{Total}$	$BSAF_{Ca(NO3)2}$
		kg_s kg dry $weight_w^{-1}$	kg_s kg dry $weight_w^{-1}$
Bernow	B	0.41	1.21
Canisteo	A	0.43	33.5
Dennis	A	0.43	1.54
Dennis	B	0.38	7.99
Dougherty	A	0.48	0.88
Hanlon	A	0.45	3.44
Kirkland	A	0.40	0.96
Luton	A	0.29	16.8
Mansic	A	0.43	21.1
Mansic	B	0.43	52.0
Norge	A	0.22	0.40
Osage	A	0.32	2.88
Osage	B	0.31	4.00
Pond Creek	A	0.39	0.90
Pond Creek	B	0.35	1.87
Pratt	A	0.52	0.95
Pratt	B	0.55	1.57
Richfield	B	0.35	6.95
Summit	A	0.47	27.8
Summit	B	0.36	5.90
Taloka	A	0.46	24.1
Teller	A	0.13	0.21
MINIMUM		0.13	0.21
MAXIMUM		0.55	52.0
MEAN		0.39	9.86
MEDIAN		0.40	3.16

[a] $BSAF_{Total}$ calculated as mg Zn kg dry weight $worm^{-1}$:Total mg Zn kg dry weight $soil^{-1}$.
[b] $BSAF_{Ca(NO3)2}$ calculated as mg Zn kg dry weight $worm^{-1}$:$Ca(NO_3)_2$-extractable mg Zn kg dry weight $soil^{-1}$.

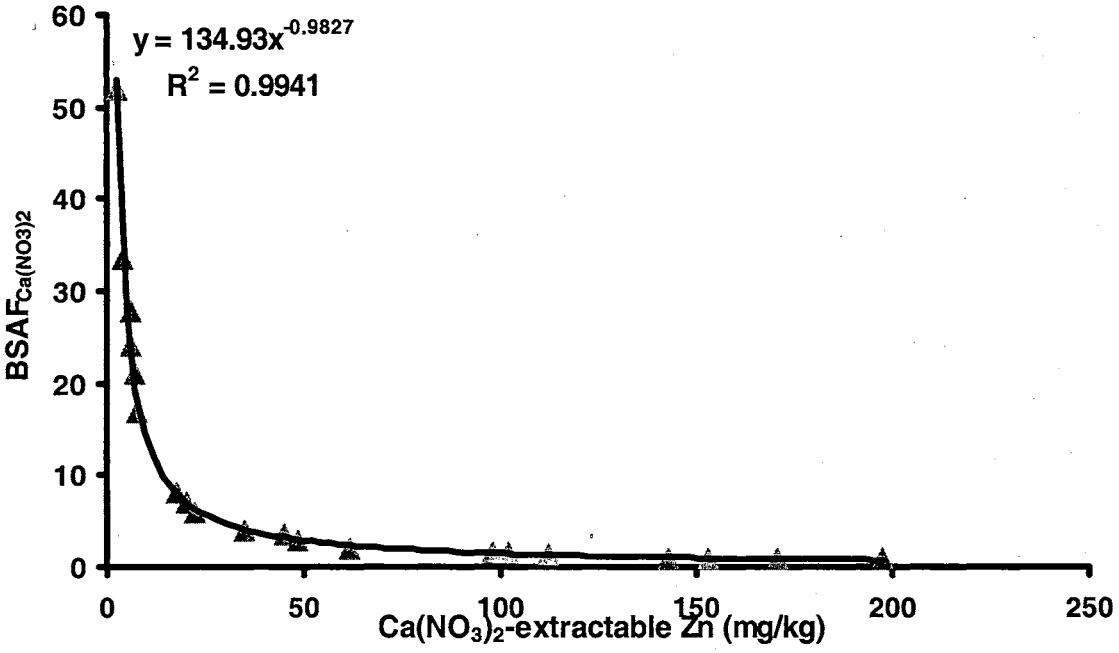

Figure 14. The biota-soil accumulation factors (BSAFs) as a function of $Ca(NO_3)_2$-extractable Zn concentrations.

CHAPTER 4

EFFECT OF SOIL PROPERTIES ON THE BIOAVAILABILITY AND TOXICITY OF ARSENIC TO *EISENIA ANDREI*

ABSTRACT

As bioavailability and toxicity is directly influenced by soil properties. In the present study, the relationship between soil properties, and As bioavailability and toxicity in earthworms (*Eisenia andrei*) exposed to 22 field soils spiked with As is investigated to develop a mathematical model to describe this relationship. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to the same total As concentration in spiked soils. Statistical models were developed for earthworm mortality, cocoon production, internal concentrations, extractable As levels, and bioaccumulation factors. Soil pH and clay were found to be the most important soil properties modulating As bioavailability for bioaccumulation factors and extractable As levels ($P < 0.05$). Correlation analysis established a significant relationship ($P < 0.01$) for pore water extractable As and mortality. In soils with the largest bioaccumulation factors, little or no mortality was observed suggesting that bioaccumulation factors are poor indicators of As toxicity to earthworms.

INTRODUCTION

Arsenic (As) contamination of soil is a worldwide problem that may pose a risk to soil organisms. Sources of As contamination of soil include mining and smelter

operations, industrial discharge, pesticides, herbicides, and wood preservatives (Adriano, 2001). In order to protect and/or restore soil ecosystems, it is necessary to accurately characterize the risk posed to soil organisms by As and other contaminants. Risk is directly related to As bioavailability, which depends upon As concentration, the geochemical forms of As, the species of organism exposed, physical and chemical characteristics of the exposure environment, and the exposure duration. Bioavailability and toxicity are not permanent properties of soil but vary with the interaction between the soil and the organism (Lanno and McCarty, 1997). There are direct and indirect methods for determining bioavailability. Direct measures of bioavailability incorporate organismal responses and/or internal chemical concentrations to estimate bioavailability. Indirect measurements of bioavailability do not use living organisms to estimate the bioavailability of chemicals from soil. Instead, they utilize measured concentrations of chemical species that are potentially available for uptake. Weakly bound or available metals are believed to be available for uptake by earthworms (Posthuma et al., 1997; Peijnenburg et al., 1999). The authors suggested field soils should be tested to further validate the use of extractions as surrogate measures of bioavailability. Only an organism can determine bioavailability or toxicity (Lanno and McCarty, 1997). However, it is necessary to integrate chemical (indirect) and biological (direct) measures to accurately reflect the bioavailability of As in soils and to protect ecological receptors.

Soil protection guidelines based on total As levels are currently being developed for the protection of ecological receptors (USEPA, 2000). Due to soil modifying factors, total As concentrations are inaccurate for predicting soil organism toxicity (Peijnenburg, 1999; Meharg, 1998; McLean and Bledsoe, 1992). Risk to soil organisms based on total contaminant concentration is not an accurate predictor of adverse effects (Peijnenburg et al., 1999) because exposure expressed as total As does not consider the effects of soil modifying factors on As bioavailability. Soil properties influencing As availability and toxicity include pH and organic matter content (Meharg et al., 1998). Due to modifying factors, soil metal is often less than 100% available for uptake by organisms (Conder and Lanno, 2000). The objective of this study was to examine the effect of soil properties (e.g., pH, organic matter content, clay content), on As bioavailability and toxicity in earthworms (*Eisenia andrei*) and develop a mathematical model to describe this relationship.

METHODS AND MATERIALS

Soil collection and preparation were conducted using standard soil testing methods (See Schroder et al., in preparation, for complete methods). Twenty-two soils with diverse paleoclimatology and geology were collected from Oklahoma and central Iowa to obtain Mollisols with a high organic C content. Soils were spiked with reagent grade Na_2HAsO_4 to obtain soil concentrations of approximately 250 mg As kg^{-1}. Spiked soils were subjected to four wet-dry cycles (see Schroder et al., in preparation, for more detail) to achieve adequate

reaction with the soil matrix and reduce the "salt effect" where heavy metal availability is greater in spiked soil than aged contaminated soil with similar metal contamination (Logan and Chaney, 1983). Total As in collected soils was determined by an acid digestion microwave technique according to U.S. EPA Method 3051 for confirmation of background As concentrations prior to analysis of chemical and physical properties (U.S. EPA 1994). Soil pH was determined in 1:1 soil:water suspension (Thomas, 1996). Soil organic C content was determined by acid dichromate digestion according to Heanes (1984). Cation exchange capacity of non-calcareous soil (soil pH < 7.0) was determined using a procedure adopted from Hendershot and Duquette (1986). Cation exchange capacity of calcareous soils (soil pH > 7.0) was determined according to the method of Polemio and Rhoades (1977). Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). Pore water extractions were determined by placing 40.0 g of soil into plastic solo cups, adding deionized distilled water to make a slurry, and allowing soils to equilibrate for 48 h. The slurry was then transferred to 50 mL tubes, centrifuged at 10,000 rpm for 15 min., filtered through a 0.45 μm membrane filter, acidified with trace metal HCl, and retained for subsequent metal analyses by ICP-AES. Spiked soils were digested by microwave according to U.S. EPA Method 3051 to determine total As concentrations. Blanks, spikes and certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) were digested and analyzed for quality assurance and quality control in the determination of metal content in soil.

Earthworm bioassays

Twenty-eight day bioassays using *Eisenia andrei* were conducted with field soils spiked with 250 mg As kg^{-1}. The bioassays were performed in triplicate for each soil-As combination and conducted using mature (clitellate) manure worms (*E. andrei*) according to a standard protocol (American Society for Testing and Materials, 1997). The 200-g soil samples were moistened and maintained between 1/3 bar and saturation, placed in glass jars with 3 small air holes in the lid, and acclimated in an environmental chamber maintained at 20±1°C for 24 h prior to the addition of 10 earthworms per replicate. Twenty-four hours prior to the addition of earthworms to test soils, mature (clitellate) earthworms weighing approximately 0.2-0.4 g were removed from synchronized in-house cultures, rinsed with reagent grade water, and placed on moist filter paper for 24 hours to depurate most of the bedding material from their intestinal tracts (Van Gestel et al. 1993). At the start of the toxicity test, randomly chosen earthworms were removed from the filter paper, rinsed, and separated into replicates of 10 earthworms. Each replicate was blotted dry, weighed, and transferred to one of three jars prepared for each soil. Testing was conducted in an environmental chamber maintained at 20 ± 1°C with constant light. Earthworms were monitored after six hours for physical condition and to determine if burrowing had occurred. Earthworms were observed daily for the first eight days and three times a week thereafter for the remainder of the test to assess the general condition of the worms and remove mortalities. Cocoons were collected on a daily basis by hand sorting. Simultaneously, observations on earthworm performance in ASTM

artificial soil (American Society for Testing and Materials, 1997) and unspiked reference soils from each site served as controls for quality assurance with respect to survival, cocoon production, and growth. Artificial soils consisted of 69.5% silica sand, 20% kaolin clay, 10% 2-mm sieved *Sphagnum* peat moss, and approximately 0.5% $CaCO_3$ added to adjust the pH to 7.0±0.5. Reagent grade water was added to hydrate the artificial soil to 45% of its dry weight. All soil materials used were hydrated and allowed to acclimate in the environmental chamber maintained at 20±1°C 24 hours prior to the start of the tests. Earthworms were judged dead if no response was observed after gentle stimulation with a blunt probe. Dead earthworms were removed, rinsed thoroughly with reagent grade water, individually wrapped in aluminum foil, and frozen at -20°C for subsequent analysis. At day 28 of each study, live earthworms were depurated for 24 hours on moist filter paper, rinsed, weighed, and stored as described above. Upon the completion of toxicity tests, individual soil replicates from all experiments were stored at -20°C in Ziploc® freezer bags.

Internal concentrations

Earthworm As concentrations were determined as described by Cervera et al. (1993), with noted exceptions. An individual worm from each replicate (3 replicates per soil-As combination) was removed from the freezer, dried for 24 hours at 80°C in a pre-weighed crucible, and weighed. All reagents used were of the highest purity available from Fisher Scientific. Individual worms were then wet digested using 5 mL each of 20% (w/v) $Mg(NO_3)_2$ and concentrated trace

metal grade HNO_3 (Fisher Scientific), covered with a watch glass, and allowed to reflux overnight at 80°C. After evaporation to total dryness (8-12 h) has been achieved, samples were ashed in a muffle furnace at 450°C for 12-14 h or until ash was completely white. The white ash was dissolved in 2 mL each of reagent grade water and concentrated trace metal grade HCl, and heated for 15 minutes at 60°C. Before diluting to a final volume of 10 mL with 3 *M* HCl, 250 μL of KI solution containing 40% (w/v) KI and 4% (w/v) ascorbic acid was added. Worm digests were stored in Nalgene® low-density polyethylene bottles until analysis. As concentrations in digests were measured using hydride generation inductively coupled plasma atomic emission spectroscopy (HG-ICP-AES). The limit of detection for As in earthworm tissue digests was 0.7 μg/L. As concentrations in worm tissues were expressed on an mg kg^{-1} dry weight basis. All analyses included procedural blanks, spikes, and certified reference material (National Institute of Standards and Technology Reference Material Oyster Tissue SRM 1566b). Mean (%RSD) spike and certified reference material recoveries were 96 (1.4%) and 94 (2.7%), respectively.

Data analysis

Statistical analyses were performed using PC SAS Version 8.2 (SAS Institute Inc., Cary, NC). LC_{50} values were based on models produced by Proc Probit. Empirical models were developed for comparison to models in the literature. Backwards-stepwise regression analysis was used to derive empirical models capable of predicting effects of As on earthworm mortality, internal

concentrations, and cocoon production based on soil properties. The backwards-stepwise regression analysis was used to identify critical soil properties explaining most of the variation. Soil properties that did not explain a significant part of the variation ($P > 0.10$) were not used as independent variables in the multiple regression equation. Statistical models capable of predicting effects of As bioavailability, based on soil properties were obtained from the regression analysis. The multiple regression functions have the format:

$$Y = b_0 + b_1(pH) + b_2(clay) + b_3(OC)$$

Where Y = extractable As, earthworm response (mortality, internal concentrations, and cocoon production), or biota-soil accumulation factors, A, B, and C = soil properties (pH, clay, OC), and a, b, and c = coefficients.

Empirical models were compared with quantitative causal values for each soil property provided by path analysis models. Path analysis, an extension of the regression model, is a statistical technique that differentiates between correlation and causation (Basta et al., 1993). Path analysis was used to decompose correlations in the model into direct or causal effects of soil properties (Loehlin, 1987) on earthworm mortality, internal concentrations, and cocoon production. Path analysis allows the partitioning of simple correlation coefficients between dependent (e.g. mortality) and independent variables (soil properties) into direct and indirect effects (Basta et al., 1993). Path analysis also provides a numerical value for each direct and indirect effect and indicates the relative strength of that correlation or causal influence (Loehlin, 1987). Direct effects are standardized

partial regression coefficients designated as path coefficients (Basta et al., 1993). Direct and indirect effects are derived from multiple linear regression of soil properties on earthworm response (mortality, internal concentrations, and cocoon production) and simple correlation values between soil properties. In addition, an uncorrelated residual (U) was determined from this model using the following equation:

$$U = \sqrt{1 - R^2}$$

A path analysis model was composed to study the effect of pH, OC, and clay on earthworm mortality (Figure 1). Direct effects (path coefficients) of soil properties on earthworm mortality are represented by the single-headed arrows while the double-headed arrows represent intercorrelation coefficients. Indirect effects of soil properties on earthworm mortality are determined from the product of one double-headed arrow and one single-headed arrow (Basta et al., 1993). Path analysis results were derived using the following equations (Williams et al., 1990):

$$r_{14} = P_{14} + r_{12}P_{24} + r_{13}P_{34} \qquad [1]$$

$$r_{24} = r_{12}P_{14} + P_{24} + r_{23}P_{34} \qquad [2]$$

$$r_{34} = r_{13}P_{14} + r_{23}P_{24} + P_{34} \qquad [3]$$

where r_{i4} corresponds to the simple correlation coefficient between the soil property and earthworm response, P_{i4} are path coefficients (direct effects) of soil property i on earthworm response, and $r_{ij}P_{i4}$ are the indirect effects of soil property j through i on earthworm response. Subscript designations are: (1) pH, (2) OC, (3) Clay, and (4) earthworm response.

The path analysis results can be presented in a concise table (Williams et al., 1990). This table provides underlined diagonal numbers indicating direct effects and off-diagonal numbers indicating indirect effects. The position of each response in the table corresponds to its position in the matrix of respective equations (equations [1], [2], [3], above). This format allows all potential tables to be presented as one table.

RESULTS AND DISCUSSION

Metal availability

The 22 soils collected had a wide range of soil properties including soil pH (4.0–8.0), cation exchange capacity (3.0 to 32.4 $cmol_c\ kg^{-1}$), organic C (0.3 to 3.0%), and clay content (5.0 to 71%) (Table 22). The As content of collected soils was similar to uncontaminated background soil contents prior to As amendment (See Schroder et al., in preparation). The target value for As amended in the test soils was 250 mg kg^{-1}, based upon earthworm responses in range-finder tests. The mean total As content in test soils of 226 mg kg^{-1}, slightly lower than the target spike content of 250 mg kg^{-1}, was attributed to loss of soluble As during preparation of spiked soils (Table 23). The total As concentration of test soils ranged from 149 to 265 mg kg^{-1}. The mean pore water extractable As concentration was 20.8 mg L^{-1} (Table 23). According to Adriano (2001), normally less than 5% of total As is water soluble. The range of pore water extractable As

concentrations in this study suggests that soil properties modulated extractable As levels.

Extraction techniques using pore water estimate metal levels adsorbed to soil components or in minerals with high water solubility. These forms of extractable metal are a more accurate measure of actual metal exposure than total metal levels (Lanno et al., 1999). This type of extraction technique has shown promise as a toxicity-related measure of As bioavailability in soils (Deuel and Swoboda, 1972; Peijnenburg et al., 1999).

Because surrogate measures of bioavailability must be correlated with organismal responses (Lanno et al., 2002, in press), models were developed using both direct (internal concentrations) and indirect approaches (organismal responses and pore water extractions) for assessing As bioavailability and toxicity. Models were developed for earthworm mortality, cocoon production, internal concentrations, extractable As, and bioaccumulation factors. Path analysis results for soils tested are listed in Table 24. Simple correlation coefficient (*r*) values between pH, OC, clay, and extractable As concentrations are listed for comparison with path analysis results. Path analysis partitions each *r*-value into one direct effect (underlined, main diagonal positions) and two indirect effects (off diagonal positions). Path analysis models based on iron and aluminum oxides resulted in low R^2 and high U values. Models based on clay are reported due to higher R^2 and lower U values than the iron and aluminum

oxide models. Significant direct effects are indicated by corresponding *p*-values for each model tested. The model explains the variation observed in pore water extractable As ($R^2 = 0.40$, $P < 0.05$). Simple correlation results (r-values) indicate that that clay affected pore water extractable As ($P < 0.05$). Path analysis direct effects also imply that soil clay affected pore water extractable As ($P < 0.05$).

Regression analysis results for the 22 As spiked soils tested are listed in Table 25. Backwards-stepwise regression was used to identify the critical soil properties that explain most of the variation of these parameters in 22 field soils. Backwards stepwise regression results indicate that pH and clay, along with pH-OC and OC-clay interactions, explained the variance among pore water extractable As concentrations ($R^2 = 0.73$, $P < 0.01$) and were predictive of available As in soil.

Earthworm mortality

Cumulative mean (n = 66) earthworm mortality was 1.2%, which was < 10% in each of the 22 unspiked reference soils. Earthworm mortality ranged from zero mortality to complete acute mortality when exposed to As spiked soils (Figure 17). Adverse physiological responses to As exposure included dermal lesions and yellow secretions, typical of stress responses in *E. andrei* (Edwards and Bohlen, 1992). In soils with 100% mortality (Norge A and Pratt B), earthworms died during the first week of the experiment. This was the case for Norge A and Pratt B soils which had 80% and 53% mortality, respectively, by day 3. In Norge

A and Pratt B, all worms were dead by day 4 and day 6, respectively. The range of mortality observed in our study was the result of differences in As bioavailability due to As interactions with the soil properties, assuming similar behavior of earthworms in each soil. Estimated LC_{50} for probability of earthworm mortality based pore water extractable As is 60.1 mg As L^{-1} (Figure 18). Correlation analysis found a significant relationship between pore water extractable As and mortality ($P < 0.01$).

Results indicate that the model did not adequately explain the variability in mortality ($R^2 = 0.34$) and could not be used with confidence to explain the variability in mortality (Table 24). However, complete (100%) mortality was only observed in two of the 22 soils, which may contribute to the low R^2 value. Simple correlation results (*r*-values) indicate that soil pH significantly affected mortality ($P < 0.01$). The path analysis partitioning shows pH ($P < 0.05$) on mortality. Backwards-stepwise regression results reveal that clay was most significant in modifying As bioavailability for mortality ($R^2 = 0.34$, $P < 0.01$) (Table 25). Clay and pH have been reported in regression models as the most important soil properties modifying As uptake rates (Peijnenburg et al. 1999, Janssen et al., 1997).

Internal concentrations

The mean (SD) internal concentration of *E. andrei* exposed to unspiked reference soils was 2.5 mg As kg^{-1} (1.0) (Table 26). The mean (SD) As

concentration of earthworms exposed to 250 mg As kg^{-1} was 287 mg kg^{-1} (181). A 19-fold difference in concentration in earthworms exposed to the same total soil content of As in 22 soils indicates that soil properties are modifying the uptake of As. This is consistent with the findings of Peijnenburg et al. (1999), indicating that soil properties have a significant impact on As uptake by *E. andrei*.

Earthworms exposed to soils where 100% mortality occurred were removed from internal As concentration statistical models to avoid comparison of depurated and non-depurated worms because there may be differences in the soil content of the earthworm gut and differences in As uptake due the physiological effects of acutely toxic As exposure. Results indicate that the model did not adequately explain the variability in internal concentrations (R^2 = 0.24) and could not be used with confidence to explain the variability in internal concentrations (Table 24). Backwards-stepwise regression results did not adequately explain the variance among internal concentrations (R^2 = 0.29, P = 0.07) (Table 25). In a previous study, regression formulae suggest the most significant impact on As uptake is pH and clay and that As is primarily taken in via the labile (extractable) soil fraction (Peijnenburg et al. 1999). Results established a non-significant relationship for pore water extractable As and internal As concentrations (P = 0.99).

Earthworm detoxification mechanisms for As are not as well studied as that of Cd or Pb. According to Morgan et al. (1994), arsenic was found in association with

sulphur in the chloragocytic compartment of earthworms and was not found in the phosphate-rich chloragosome granules. The author stated that arsenic does not accumulate in the form of arsenate in the chloragosomal tissues even if taken up by earthworms as a phosphate analogue. Adverse effects can be expected only when the capacity of detoxification mechanisms is exceeded (Lock, 2001). Resulting mortalities in Norge A and Pratt B suggests that detoxification mechanisms were exceeded. A non-significant relationship was found for internal concentrations and mortality ($P = 0.37$).

Cocoon production

The absence of a relationship for cocoon production of *E. andre*i and internal concentrations was observed (Figure 19). Results indicate that the model did not adequately explain the variability in cocoon production ($R^2 = 0.16$) and could not be used with confidence to explain the variability in cocoon production (Table 24). Backwards-stepwise regression results found the relationship between soil properties and cocoon production non-significant ($P = 0.15$) (Table 25).

Biota-soil accumulation factors

Biota-soil accumulation factors were developed for this study based on total As concentrations ($BSAF_{Total}$) and pore water extractions ($BSAF_{PW}$). Biota-soil accumulation factors represent the ratio of internal As concentrations in exposed earthworms to concentrations in the exposure matrix. Calculated $BSAF_{Total}$ and $BSAF_{PW}$ values are listed in Table 27. Biota-soil accumulation factors ranged approximately 18-fold from 0.15 – 2.7 for $BSAF_{Total}$ and over four orders of

magnitude from 0.6 – 1.2E+04 for $BSAF_{PW}$. $BSAF_{PW}$ values decreased with increasing extractable As concentrations (Figure 20). Results are consistent with previous findings of bioaccumulation factors based on total As decreasing with increasing soil As concentrations (Meharg, et al., 1998).

Non-significant coefficient of determination (R^2) values and high uncorrelated (U) values indicate that the model did not adequately explain the variability in the biota-soil accumulation factors and could not be used with confidence to explain the variability in $BSAF_{PW}$ (Table 24). Backwards-stepwise regression results suggest that clay, in addition to pH-OC, pH-clay, and OC-clay interactions, is significant in explaining the variance in $BSAF_{PW}$ ($R^2 = 0.66$, $P < 0.01$) (Table 25). Previous earthworm bioaccumulation factor based on total As have shown that pH and organic matter content affected As bioaccumulation and bioavailability (Meharg, et al., 1998). The regression model developed in this study is consistent with this finding.

Bioaccumulation factors are frequently used to assess the effect of soil-modifying factors on the bioavailability of metals in soils (Janssen et al., 1997; Peijnenburg et al., 1999). However, there is much debate about the use of bioaccumulation factors to assess the bioavailability of metals. Some authors report that bioaccumulation factors should be questioned because they tend to decrease with increasing metal concentrations, indicating there is no relationship between

the internal concentration and the bioavailable concentration (Lock, 2000). Others state that bioaccumulation factors are more appropriate than body concentrations for normalization among field soils (Janssen, 1997). Bioaccumulation factors are usually normalized to total contaminant concentrations in soil. Peijneburg et al. (1999), suggests bioaccumulation factors should be based on bioavailable concentrations in the soil. In this study, bioaccumulation factors based on pore water extractable As were found to decrease with increasing concentrations of available As. It is assumed that bioaccumulation occurs when BSAFs are greater than one (Lock, 2001). Many of the bioaccumulation factors based on total As in this study were less than unity. However, bioaccumulation factors based on bioavailable concentrations in this study found many of the values were greater than one. In soils with the largest $BSAF_{PW}$ values, little or no mortality was observed suggesting that $BSAF_{PW}$ are poor indicators of adverse effects of As to earthworms ($P = 0.17$).

Empirical formulas developed may be useful for predicting the potential environmental risks of As in soil. However, path analysis models did not prove useful for providing a quantitative causal influence of As bioavailability and toxicity to earthworms. Conclusions of our study support the use of poor water extractions as a surrogate measure of the adverse effect of As to earthworms. The non-significant relationship found for internal concentrations and mortality suggests that internal concentrations may not prove useful as indicators of adverse effects of As toxicity and bioavailability to earthworms. Biota-soil

accumulation factors in this study were deemed as poor indicators of environmental risk of As toxicity. Furthermore, the decrease in $BSAF_{PW}$ with increasing available As concentrations indicates that BSAFs should not be used assess the influence of soil properties on As bioavailability.

Acknowledgements

We would like to thank Derrick Chappell for assistance with bioassays. The research in this manuscript has been funded by the U.S. Environmental Protection Agency, Office of Research and Development, National Center for Exposure Assessment. Although the research described in this paper has been funded by the U.S. EPA to Dr. Roman Lanno and Dr. Nicholas Basta, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

Adriano, DC. 2001. *Trace elements in terrestrial environments.* Springer, New York, USA.

American Society for Testing and Materials. 1997. Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with Lumbricid earthworm *Eisenia fetida*, standard E 1676-97. In: Annual Book of ASTM Standards, pp. 1056-1074.

Basta NT, Pantone DJ, Tabatabai MA. 1993. Path analysis of heavy metal adsorption by soil. *Agron. J.* 85:1054-1057.

Cervera ML, Navarro A, Montoro R, Gomez J. 1993. Inductively coupled plasma atomic emission spectrometric determination of arsenic in mussel products. *Fresenius J Anal Chem* 347:58-62.

Conder JM, Lanno RP. 2000. Evaluation of cadmium, lead, and zinc to *Eisenia fetida*. *Chemosphere*. 41:1659-1668.

Edwards CA, Bohlen PJ. 1992. The effects of toxic chemicals on earthworms. *Rev. Environ. Contam. Toxicol.* 125:23-99.

Gee GW, Bauder JW. 1986. Particle-size analysis. In A. Klute, ed., *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods,* 2nd ed. Agronomy Monograph 9. Soil Science Society of America, Madison, WI, USA, pp. 383-411.

Heanes DL. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Commun. In Soil Sci. Plant Analysis.* 15:1191-1213.

Hendershot WH, Duquette M. 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Sci. Soc. Am. J.* 50:605-608.

Janssen RPT, Posthuma L, Baerselman R, Den Hollander HA, Van Veen RPM, Peijnenburg WJGM. 1997. Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal accumulation in earthworms. *Environ. Toxicol. Chem.* 16:2479-2488.

Lanno R. Ed. 2002 (In press). *Contaminated soils: From soil-chemical interactions to ecosystem management. Proceedings of Workshop on Assessing Contaminated Soils, Pellston, MI, 23-27 September 1998.* Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, USA.

Lanno RP, Conder JM, Seals L. 1999. Critical limits for heavy metals in soils and surface waters. *Workshop on effects-based approaches for heavy metals.* Federal Environmental Agency (Umweltbundesamt), Berlin, October.

Lanno RP, McCarty LS. 1997. Earthworm bioassays: Adopting techniques from aquatic toxicology. *Soil Biol. Biochem.* 29:693-697.

Lock K, Janssen CR. 2001. Zinc and cadmium body burdens in terrestrial Oligochaetes: use and significance in environmental risk assessment. *Environ. Toxicol. Chem.* 20:2067-2072.

Lock K, Janssen CR, De Coen WM. 2000. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to *Enchytraeus albidus*. *Environ. Toxicol. Chem.* 19:2666-2671.

Loehlin JC. 1987. *Latent variable models: an introduction to factor, path, and structural analysis.* Lawrence, Hillsdale, New Jersey, USA.

McLean LE, Bledsoe BE. 1992. Ground water issue: Behavior of metals in soils. EPA/540/S-92/018. U.S. Environmental Protection Agency, Washington, DC.

Meharg AA, Shore RF, Broadgate K. 1998. Edaphic factors affecting the toxicity and accumulation of arsenate in the earthworm *Lumbricus terrestris*. *Environ. Toxicol. Chem.* 17:1124-1131.

Morgan AJ, Winters C, Yarwood A. 1994. Speed-mapping of arsenic distribution in the tissues of earthworms inhabiting arsenious soil. *Cell Biol. Int.* 18:911-914.

Peijnenburg WJGM, Baerselman R, de Groot AC, Jager T, Posthuma L, Van Veen RPM. 1999. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. *Ecotoxicol. Environ. Saf.* 44:294-310.

Polemio M, Rhoades JD. 1977. Determining cation exchange capacity: a new procedure for calcareous and gypsiferous soils. *Soil Sci. Soc. Am. J.* 41:524-528.

Posthuma L, Baerselman R, Van Veen RPM, Dirven-Van Breemen EM. 1997. Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. *Ecotoxicol. Environ. Safety* 38:108-121.

Schroder J. 2002 (In preparation). Ph.D. Dissertation. Oklahoma State University, Stillwater, OK.

Thomas GW. 1996. Soil pH and soil acidity. In Sparks DL, ed, *Methods of Soil Analysis*. Part 3 Chemical Methods-SSSA Book series no. 5. Soil Science Society of America, Madison, WI, pp. 475-490.

U.S. Environmental Protection Agency. 1994. Method 3051: Microwave

assisted acid digestion of sediment, sludges, soils, and oils.

U.S. Environmental Protection Agency. 2000. Ecological soil screening level guidance. Draft Report. Office of Emergency and Remedial Response. Washington, D.C.

Van Gestel CAM, Dirven-van Breeman EM, Baerselman R. 1993. Accumulation and elimination of cadmium, chromium, and zinc and the effects on growth and reproduction of *Eisenia andrei* (Oligochaeta, Annelida). *Sci. Total Environ. Supplement* 585-597.

Williams WA, Jones MB, Demment MW. 1990. A concise table for path analysis statistics. *Agron. J.* 82:1022-1024.

Table 22. Properties of Soils

Soil	Horizon	Unspiked Soil pH[a]	As spiked soil pH[a]	CEC cmol/kg[b]	OC	Sand %	Clay	Silt	Class
Bernow	B	4.9	4.5	6.74	0.30	58.8	26.3	17.5	Sandy Clay Loam
Canisteo	A	7.5	7.6	30.5	3.00	31.3	38.8	51.3	Silt Loam
Dennis	A	5.6	4.9	9.77	1.90	37.5	23.8	40.0	Loam
Dennis	B	6.1	5.6	14.6	0.80	21.3	45.0	40.6	Clay Loam
Doughtery	A	5.3	5.0	3.33	1.20	75.0	11.3	21.3	Loan Sand
Hanlon	A	7.4	7.0	16.3	1.60	63.8	17.5	23.8	Sandy Loam
Kirkland	A	5.6	5.1	14.0	1.45	12.5	31.3	57.5	Silty Clay Loam
Luton	A	7.1	7.2	32.4	2.00	2.50	71.3	38.8	Clay
Mansic	A	7.8	8.0	16.5	1.50	33.8	30.0	43.8	Loam
Mansic	B	8.0	8.0	11.7	0.53	30.0	35.0	42.5	Clay Loam
Norge	A	4.0	4.0	4.57	1.20	36.9	17.5	45.6	Silt Loam
Osage	A	6.6	6.0	28.3	2.60	13.8	55.7	53.8	Silty Clay Loam
Osage	B	6.8	6.2	27.5	2.00	11.3	61.3	47.5	Silty Clay
Pond Creek	A	5.2	4.7	10.7	1.90	16.3	28.8	62.5	Silt Loam
Pond Creek	B	6.0	6.0	12.5	0.80	18.8	32.5	48.8	Silty Clay Loam
Pratt	A	6.5	6.3	4.40	0.90	90.0	5.00	3.80	Silt
Pratt	B	6.4	6.0	3.40	0.50	92.5	6.25	1.30	Silt
Richfield	B	7.7	7.6	22.3	1.10	11.3	41.3	51.3	Silty Clay Loam
Summit	A	7.2	7.3	29.4	2.40	17.5	45.7	53.8	Silty Clay Loam
Summit	B	7.1	6.7	27.6	1.25	10.0	56.8	48.8	Silty Clay
Taloka	A	5.1	4.7	4.85	1.20	20.0	11.3	58.8	Silt Loam
Teller	A	4.5	4.3	3.01	0.85	66.9	10.0	23.8	Silt Loam
MINIMUM		4.0	4.0	3.01	0.30	2.50	5.00	1.30	
MAXIMUM		8.0	8.0	32.4	3.00	92.5	71.3	62.5	
MEAN		6.3	6.0	15.2	1.41	35.1	32.0	40.0	
MEDIAN		6.5	6.0	13.3	1.25	25.7	30.7	45.0	

[a] pH determined by 1:1 soil:water
[b] Cation Exchange Capacity measured using 0.1 M $BaCl_2$ for non-calcareous soils; 1 M NaOAc, pH 8.2 for calcareous soils.

Table 23. Soil As concentrations.

		Pore water extractable As[a]		Total As[b]	
Soil	Horizon	mg L^{-1}	%RSD	mg kg^{-1}	%RSD
Bernow	B	0.19	3.21	255	0.17
Canisteo	A	8.44	0.33	225	0.56
Dennis	A	0.30	12.2	234	2.30
Dennis	B	0.00	26.2	222	4.41
Dougherty	A	98.8	2.04	205	6.10
Hanlon	A	13.9	0.92	228	3.42
Kirkland	A	2.01	0.77	226	0.12
Luton	A	1.49	0.86	242	1.01
Mansic	A	16.9	1.18	201	1.60
Mansic	B	20.3	3.52	210	2.13
Norge	A	4.06	1.90	223	0.07
Osage	A	0.23	0.12	265	1.68
Osage	B	0.12	5.86	234	2.43
Pond Creek	A	7.49	1.09	227	3.77
Pond Creek	B	0.49	4.55	228	1.91
Pratt	A	97.4	0.70	149	4.32
Pratt	B	163	2.48	218	7.33
Richfield	B	8.61	0.89	234	0.49
Summit	A	0.54	1.85	258	0.97
Summit	B	0.02	0.00	243	2.85
Taloka	A	3.81	0.46	208	6.75
Teller	A	10.1	1.85	229	4.22
MINIMUM		0.00	0.00	149	0.07
MAXIMUM		163	26.2	265	7.33
MEAN		20.8	3.32	226	2.66
MEDIAN		3.94	1.51	227	2.21

[a] Extracted using distilled deionized water, mean (n=2)
[b] Extracted according to EPA Method 3051 and measured by ICP-AES, mean (n=2)

Table 24. Path analysis direct effects (diagonal, underlined) and indirect effects (off diagonal) of soil pH, organic carbon (% OC), and clay (mmol kg^{-1}) on extractable As concentrations and *Eisenia andrei* after 28-day exposure to As.

Response		pH	OC	Clay	r	R^2	U
Pore water extractable (mg As L^{-1})	pH	0.31	-0.04	-0.29	-0.01	0.40*	0.77
	OC	0.08	-0.14	-0.30	-0.36		
	Clay	0.14	-0.07	-0.63*	-0.55**		
% Mortality	pH	-0.08	0.00	-0.25	-0.32	0.34	0.81
	OC	-0.02	0.02	-0.27	-0.27		
	Clay	-0.03	0.01	-0.55*	-0.58**		
Internal concentration[a] (mg/kg dry wt.)	pH	0.48	0.04	-0.11	0.40	0.24	0.87
	OC	0.10	0.18	-0.12	0.16		
	Clay	0.19	0.08	-0.28	-0.02		
Cocoon Production[a]	pH	-0.26	0.09	0.05	-0.12	0.16	0.92
	OC	-0.07	0.33	0.06	0.32		
	Clay	-0.12	0.16	0.11	0.16		
$BSAF_{PW}$[b]	pH	-0.22	-0.09	0.23	-0.09	0.30	0.84
	OC	-0.05	-0.41	0.25	-0.21		
	Clay	-0.09	-0.18	0.57*	0.31		

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Cumulative (mean of 3 replicates per soil-As combination) cocoons

[b] Soils with 100% mortality removed from model

Table 25. Multiple regression formulae describing the quantitative relationship between soil properties, *Eisenia andrei* after 28-day exposure to As, and extractable As concentrations.

Response	Regression equation obtained[a]	Statistics
Pore water extractable (mg As L^{-1})	$y = -331 + 141(pH) - 4.35(clay) - 9.35(pH)^2 - 11.5(pH*OC) + 1.86(OC*clay)$	$R^2 = 0.73$, n = 22, $P < 0.01$
% Mortality	$y = 49.4 - 0.88(clay)$	$R^2 = 0.34$, n = 22, $P < 0.01$
Internal concentration[b] (mg As kg dry $wt.^{-1}$)	$y = 66.5 - 103(OC)^2 + 54.2(pH*OC)$	$R^2 = 0.29$, n = 20, $P = 0.07$
Cocoon Production[b]	$y = -2.21 + 3.91(OC)$	$R^2 = 0.10$, n = 20, $P = 0.15$
$BSAF_{Ca(NO3)2}$[b]	$y = -9040 + 729(clay) + 3.98(clay)^2 + 1290(pH*OC) - 80.9(pH*clay) - 267(OC*clay)$	$R^2 = 0.66$, n = 20, $P < 0.01$

[a] All variables in the models are significant at the 0.1 level

[b] Soils with 100% mortality were removed from model

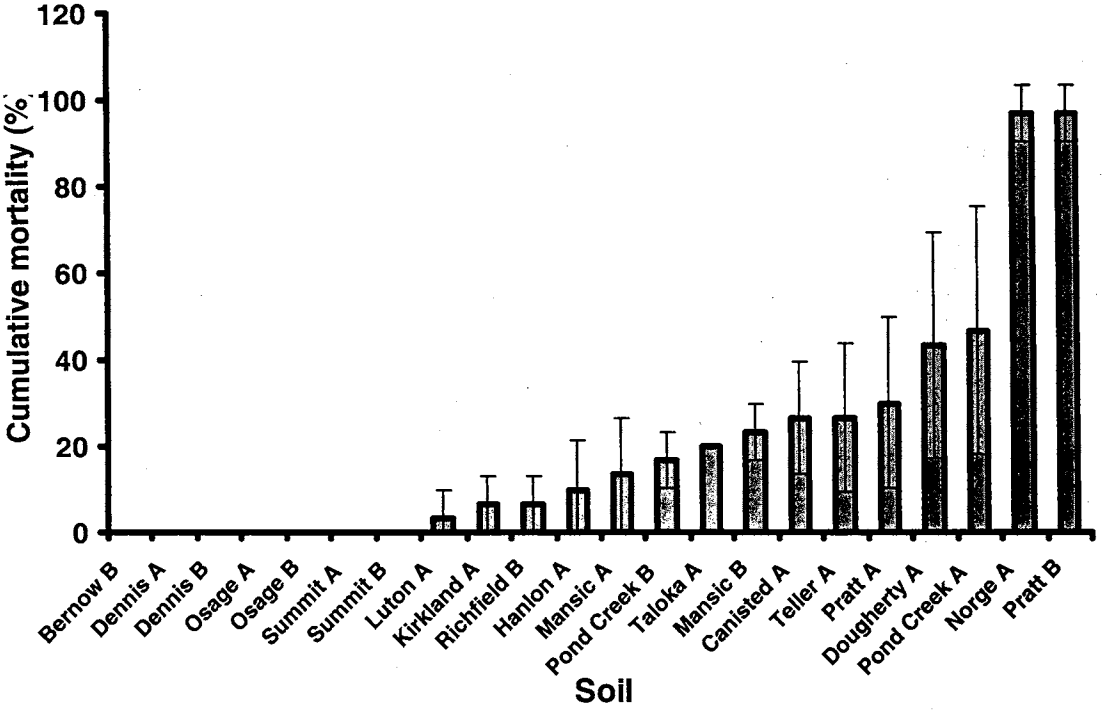

Figure 15. Cumulative mortality (mean of three replicates, ± 95% CI) of *Eisenia andrei* exposed to 250 mg As/kg spiked soils for 28 days.

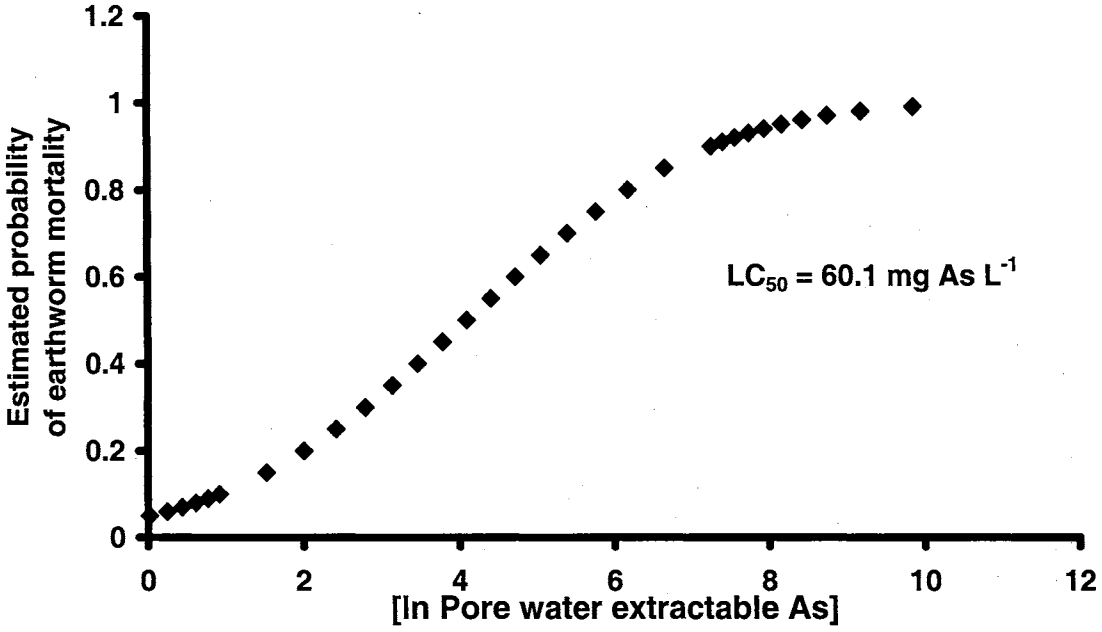

Figure 16. Estimated probability of earthworm mortality based on natural log pore water extractable As ($\chi^2 < 0.0001$).

Table 26. Internal concentrations of Eisenia andrei exposed to unspiked and As spiked soils (mean, n = 3).

Soil	Horizon	Unspiked soil earthworm concentration[1] mg kg^{-1}	%RSD	As spiked soil earthworm concentration[2] mg kg^{-1}	%RSD
Bernow	B	3.85	25.6	235	28.1
Canisteo	A	3.66	36.4	525	40.0
Dennis	A	4.42	29.6	197	12.2
Dennis	B	0.84	5.87	32.3	20.7
Dougherty	A	1.91	14.4	454	33.0
Hanlon	A	3.52	13.4	549	8.93
Kirkland	A	2.53	34.1	529	11.2
Luton	A	4.22	30.9	452	53.1
Mansic	A	4.00	48.8	440	21.9
Mansic	B	1.82	9.91	237	39.8
Norge	A	1.96	10.2	39.4	30.9
Osage	A	2.28	29.6	136	63.0
Osage	B	2.77	14.4	174	33.8
Pond Creek	A	1.18	15.2	55.8	6.10
Pond Creek	B	1.57	44.4	329	35.1
Pratt	A	2.57	7.11	59.8	22.5
Pratt	B	1.72	30.0	272	20.9
Richfield	B	2.56	26.0	629	10.8
Summit	A	1.93	28.7	317	47.8
Summit	B	1.46	35.6	129	31.4
Taloka	A	1.45	22.1	232	28.9
Teller	A	2.74	33.5	299	18.4
MINIMUM		0.84	5.87	32.3	6.10
MAXIMUM		4.42	48.8	629	63.0
MEAN		2.50	24.8	287	28.1
MEDIAN		2.40	27.3	254	28.5

[1] As concentration in digests of worms exposed to reference (unspiked) soils, measured by HG-ICP (limit of detection 0.7 µg/L)

[2] As concentration in digests of worms exposed to 250 mg/kg As spiked soils, measured by HG-ICP (limit of detection 0.7 µg/L)

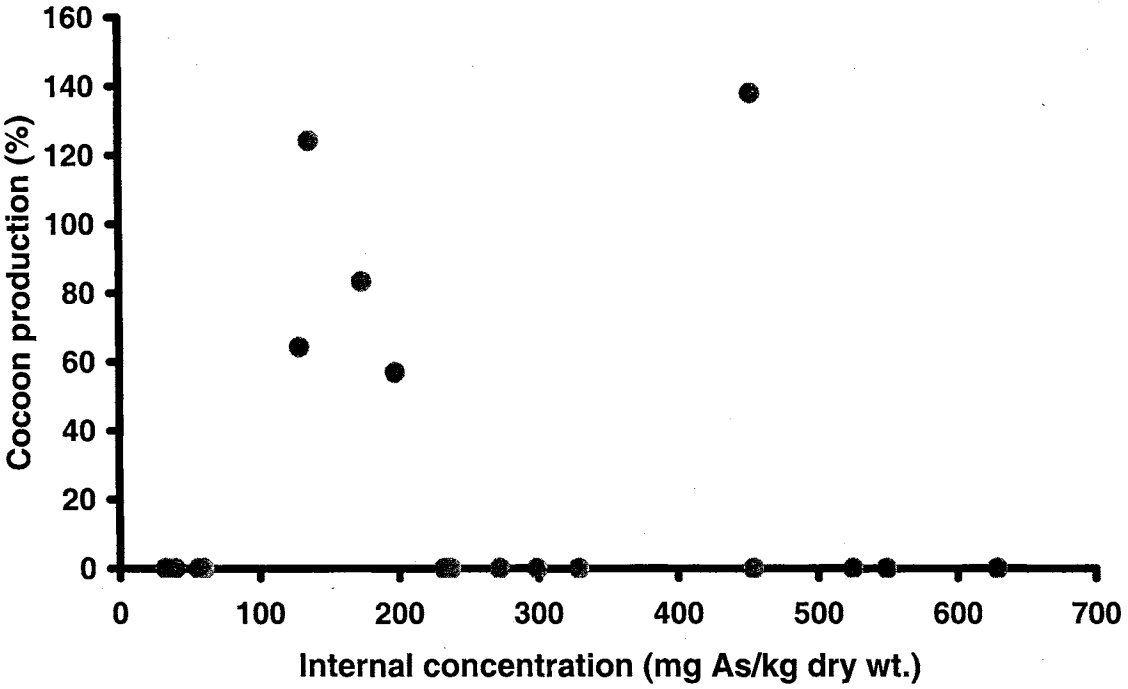

Figure 17. Cocoon production after 28-day exposure, expressed in percentage of control, versus internal concentrations.

Table 27. Biota-Soil Accumulation Factors for *Eisenia andrei* after 28-Day exposure to in 22 field soils spiked with As.

Soil	Horizon	$BSAF_{Total}$[a]	$BSAF_{PW}$[b]
		kg_s kg dry $weight_w^{-1}$	L_{PW} kg dry $weight_w^{-1}$
Bernow	B	0.92	1.3E+03
Canisteo	A	2.34	6.2E+01
Dennis	A	0.84	6.7E+02
Dennis	B	0.15	1.2E+04
Dougherty	A	2.22	4.6E+00
Hanlon	A	2.41	4.0E+01
Kirkland	A	2.34	2.6E+02
Luton	A	1.87	3.0E+02
Mansic	A	2.18	2.6E+01
Mansic	B	1.13	1.2E+01
Norge	A	0.18	9.7E+00
Osage	A	0.51	6.0E+02
Osage	B	0.74	1.4E+03
Pond Creek	A	0.25	7.4E+00
Pond Creek	B	1.44	6.7E+02
Pratt	A	0.40	6.1E-01
Pratt	B	1.24	1.7E+00
Richfield	B	2.68	7.3E+01
Summit	A	1.23	5.9E+02
Summit	B	0.53	7.4E+03
Taloka	A	1.12	6.1E+01
Teller	A	1.31	3.0E+01
MINIMUM		0.15	6.1E-01
MAXIMUM		2.68	1.2E+04
MEAN		1.27	1.2E+03
MEDIAN		1.18	6.8E+01

[a] $BSAF_{Total}$ calculated as mg As kg dry weight $worm^{-1}$:Total mg As kg dry weight $soil^{-1}$.
[b] $BSAF_{PW}$ calculated as mg As kg dry weight $worm^{-1}$:Pore water extractable mg As L^{-1}.

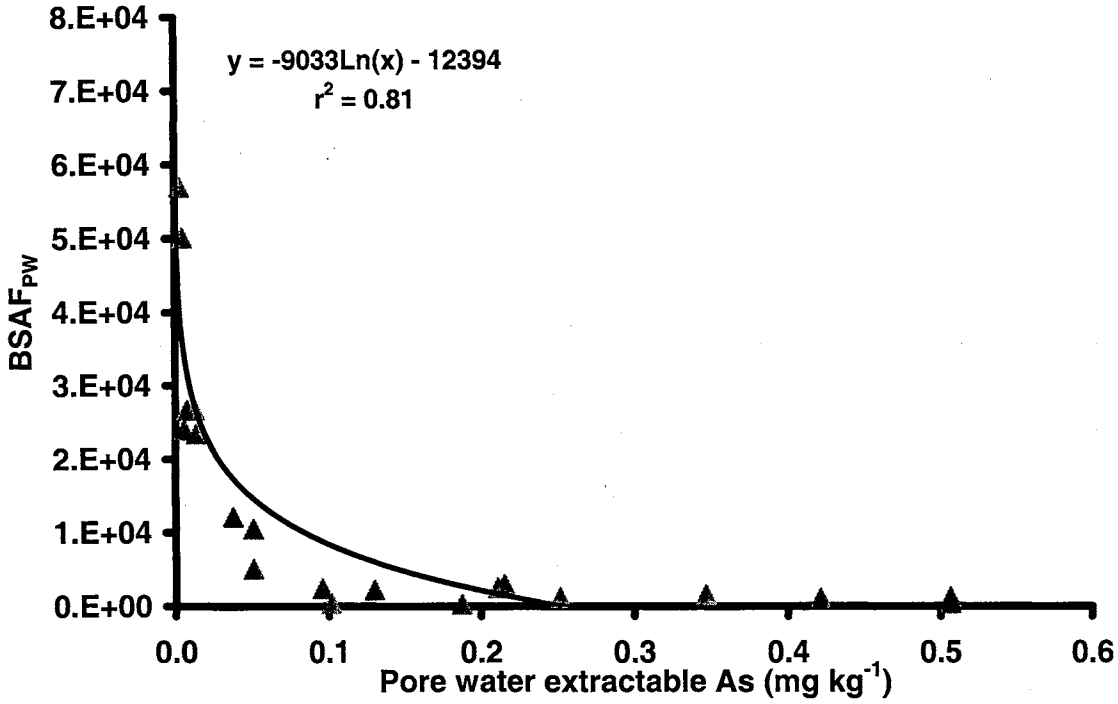

Figure 18. The biota-soil accumulation factors ($BSAF_{PW}$) as a function of pore water extractable As concentrations in spiked soils.

CONCLUSIONS

The range of mortalities observed in this study was the result of differences in metal bioavailability due to metal interactions with the soil properties, assuming similar behavior of earthworms in each soil. Path analysis models proved useful for providing quantitative causal influence of soil properties for Cd, Pb, and Zn, bioavailability and toxicity to earthworms. However, path analysis models did not prove useful for providing a quantitative causal influence of soil properties for As bioavailability and toxicity to earthworms. Overall, pH was the most important soil property modifying the bioavailability and toxicity of Cd, Pb, and Zn. This is consistent with previous findings indicating that pH is the master variable affecting metal availability.

Complete mortality (100%) was observed in soils with $Ca(NO_3)_2$-extractable Pb levels > 900 mg kg^{-1}. Results show a significant relationship ($P < 0.0001$) for $Ca(NO_3)_2$-extractable Pb and mortality. Regression analysis results established a significant relationship ($P < 0.0001$) for 0.5 M $Ca(NO_3)_2$-extractable Cd and internal Cd concentrations. Due to Zn regulation by earthworms, a relationship was not found for $Ca(NO_3)_2$-extractable Zn and internal concentrations. However, correlation analysis found the relationship between mortality and $Ca(NO_3)_2$-extractable Zn concentrations was significant ($r = 0.47$). A non-significant relationship was found for internal As concentrations and mortality ($P = 0.37$). Correlation analysis established a significant relationship ($P < 0.01$) for pore water extractable As and mortality.

Biota-soil accumulation factors in this study were deemed as poor indicators of adverse effects of metals. Furthermore, decreasing bioaccumulation factors with increasing available metal concentrations indicate that BSAFs should not be used assess the influence of soil properties on metal bioavailability. The absence of a significant relationship found for internal concentrations and mortality suggests that internal concentrations may not prove useful as indicators of adverse effects of As and Cd toxicity and bioavailability to earthworms. However, a significant relationship was found for internal concentrations and mortality suggesting that internal concentrations may prove useful as indicators of adverse effects of Pb and Zn toxicity and bioavailability to earthworms.

VITA

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