Synthesis of earthworm trace metal uptake and bioaccumulation data: role of soil concentration, earthworm ecophysiology, and experimental design

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Richardson J.B.[1], Görres J.H.[2], Tom Sizmur[3]

[1] Department of Geosciences, University of Massachusetts Amherst, Amherst MA, USA
[2] Department of Plant & Soil Science, University of Vermont, Burlington, VT 05405 USA
[3] Department of Geography and Environmental Science, University of Reading, Reading, RG6 6DW, UK

*Corresponding Author

Justin Richardson
University of Massachusetts Amherst
233 Morrill Science Center
Amherst MA 01003
Phone: 413-545-4840

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Abstract

Trace metals can be essential for organo-metallic structures and oxidation-reduction in metabolic processes or may cause acute or chronic toxicity at elevated concentrations. The uptake of trace metals by earthworms can cause transfer from immobilized pools in the soil to predators within terrestrial food chains. We report a synthesis and evaluation of uptake and bioaccumulation empirical data across different metals, earthworm genera, ecophysiological groups, soil properties, and experimental conditions (metal source, uptake duration, soil extraction method). Peer-reviewed datasets were extracted from manuscripts published before June 2019. The 56 studies contained 3513 soil-earthworm trace metal concentration paired data sets across 11 trace metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sb, U, Zn). Across all field and laboratory experiments studied, the median concentrations of Hg, Pb, and Cd in earthworm tissues that were above concentrations known to be hazardous for consumption by small mammals and avian predators but not for Cu, Zn, Cr, Ni, and As. Power regressions show only Hg and Cd earthworm tissue concentrations were well-correlated with soil concentrations with $R^2 > 0.25$. However, generalized linear mixed-effect models reveal that earthworm concentrations were significantly correlated with soil concentrations for log-transformed Hg, Cd, Cu, Zn, As, Sb ($p < 0.05$). Factors that significantly contributed to these relationships included earthworm genera, ecophysiological group, soil pH, and organic matter content. Moreover, spiking soils with metal salts, shortening the duration of exposure, and measuring exchangeable soil concentrations resulted in significantly higher trace metal uptake or greater bioaccumulation factors. Our results highlight earthworms are able to consistently bioaccumulate toxic metals (Hg and Cd only) across field and laboratory conditions. However, future experiments should incorporate greater suites of trace metals, broader genera of earthworms, and more diverse

laboratory and field settings generate data to devise universal quantitative relationships between soil and earthworm tissue concentrations.

Keywords

Bioconcentration; soil pollution; toxic metals; heavy metals; environmental toxicology

Capsule

The meta-analysis revealed that while concentrations affected earthworm Cd and Hg concentrations, earthworm properties, soil properties, and experimental design, source of metal, and exposure duration significantly affected trace metal uptake.

1. Introduction

Trace metals are generally considered to include metal and metalloid elements that occur at abundances < 0.1% of the Earth's crust (Adriano, 2001). Some trace metals, such as chromium (Cr), copper (Cu), zinc (Zn), manganese (Mn), and nickel (Ni) are essential for organisms due to their roles in folding of organo-metallic structures such as enzymes and proteins and regulating oxidation-reduction in metabolic processes (Hooda, 2010). Other trace metals, such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), antimony (Sb), and uranium (U) are non-essential and can cause acute or chronic toxicity when accumulated even in small quantities by plants and animals (Adriano, 2001; Kabata-Pendias, 2010). Trace metals are naturally present in the terrestrial environments at low concentrations but may occur at higher concentrations due to lithology (Peng et al., 2004; Maleri et al., 2008; Tijani et al., 2006) but more frequently from local and regional pollution from smelters (Morgan and Morgan, 1990; Filzek etal 2004; Dai et al., 2004; Nannoni et al., 2011), roads (Pagotto et al 2001), agricultural soil amendments

(Centofanti et al 2016), mining activities (Wang et al., 2009; Duarte et al., 2014). Urban areas with non-point source pollution can also be substantially contaminated with trace metals.

Earthworms are one of the most important soil fauna due to their size and biomass compared to other soil fauna, and their ability to physically, biologically and chemically alter soils (Scheu 1987; Bohlen et al., 2004; Sizmur and Hodson, 2009; Sizmur et al 2011). The uptake of trace metals by earthworms is of importance not only because of the potential toxicity to the earthworms themselves, but also for trophic transfer of metals from soils to predators and detritovores in terrestrial food webs (e.g. Talmage and Walton, 1993; Nahmani et al 2007; Richardson et al 2016). It is generally agreed earthworms can take up metals through ingestion of soils and dermally by exposure to dissolved metals in soil pore water (Vijver et al., 2003). Spurgeon and Hopkins (1999) showed that while earthworms were capable of regulating their internal tissue concentrations of essential metals, such as Cu and Zn, at an equilibrium level, the tissue concentrations of non-essential metals, such as Pb and Cd, do not reach equilibrium since earthworms lack specific excretion mechanisms for these elements. Regulation of tissue concentrations is also dependent on earthworm-specific physiological processes, such as the excretion of metals by the calciferous glands or retention of metals within chloragogenous tissues, where Zn and Pb are associated with ‘Type A’ phosphate-rich insoluble granules and Cu, Cd and Hg are associated with ‘Type B’ sulfur-rich metallothionein-like proteins (Fischer and Molnar 1993; Spurgeon and Hopkin 1999; Fraser et al., 2011; Karaca et al., 2010).

From the large number of studies on the bioaccumulation of trace metals by earthworms, their tissue concentrations are considered a reliable indicator of trace metal bioavailability in soils (Ma 1987; Suthar et al., 2008; Pérès et al., 2011). Linear and logarithmic equations have been developed and analyzed in aggregate to estimate uptake of metal by earthworms by

Neuhauser et al., (1995), Sample et al., (1999), and Nahmani et al., (2007). As stated by Nahmani et al., (2007) "Much work has been carried out on accumulation of metals by earthworms in soils... Yet it is still not possible to predict with a high degree of confidence the body burden of an earthworm..." The limitations to our capability to interpret and extrapolate results from studies is due to the wide range of experimental conditions, with discrepancies between laboratory conditions and the 'real world' environment. The literature contains many studies that have focused on Lumbricidae earthworms under laboratory conditions. Moreover, there are contradictions in soil to earthworm trace metal relationships reported. For example, soil concentrations of Cu, Zn, or Pb were significantly correlated with earthworm tissue concentrations ($R^2 > 0.50$) in some studies (e.g. Neuhauser, 1995; Sample et al., 1999; Ma 2004; Alvarenga et al., 2013) while others reported no significant correlation and low explanatory power ($R^2 < 0.25$) (e.g. Nahmani et al., 2007; Wang et al., 2012; Richardson et al., 2015; González-Alcaraz et al., 2018). Thus, there is a need to undertake a synthesis of literature data sets to further identify additional experimental design, soil, and earthworm properties hindering universal relationship equations with strong predictive power.

Despite a vast body of literature, there remain a number of unanswered questions about how soil properties (i.e. metal concentration, pH, organic matter) influence the bioaccumulation of metals by earthworms (Dai et al., 2004; Ma 2004; Karaca et al., 2010) and a universal quantitative relationship between soil trace metal concentrations and earthworm tissue concentrations is lacking. As a prime example, measuring bioaccumulation may be problematic because the straightforward calculation of bioaccumulation factor (BAF; synonymous with bioconcentration factor), involving the ratio of dry weight tissue concentrations by soil concentrations, can be affected by the method used to measure soil concentrations. Furthermore, experimental design artefacts or generalizations may result from the conditions under which trace metal bioaccumulation studies have been conducted. Bioaccumulation has been well-studied for a limited set of trace metals (Pb, Cu, Zn, Cd) but similar relationships may not hold true for many other trace metals. Experiments have been undertaken using a wide range of soil types, ranging from very organic rich soils to support *E. fetida* (e.g. Suthar and Singh 2009 utilized a soil composed of up to 80% cow dung) or artificial soils, such as the OECD standard soil, with <10% organic matter (Nahmani et al., 2007). Soils are frequently spiked in the laboratory with metal salts (Nahmani et al., 2007), which may not adequately represent trace metal concentrations associated with organic matter and secondary oxides of field-contaminated soils. Lastly, the duration of exposure adopted in laboratory bioassays can be far shorter than at the time expected for attenuation to occur (Sheppard et al., 1997; Nahmani et al., 2007).

The purpose of this study is to synthesise data on trace metal uptake and bioaccumulation by earthworms, similar to previous meta-analyses but include additional experimental design variables to evaluate broader patterns. This meta-analysis set out to revisit the soil-earthworm uptake paradigm in our first question and explore three additional questions centered on soil-earthworm properties and experimental design aspects. (1) To what extent are trace metals taken up and bioaccumulated by earthworms across a broad range of earthworm genera ? (2) Does soil pH, soil organic matter, genera and earthworm ecophysiological groups influence earthworm uptake and bioaccumulation of all trace metals, or only specific metals? (3) Do experimental design variables (e.g. source of metals, exposure duration) artificially influence the bioaccumulation of trace metals in earthworms? (4) Which soil extraction methods are most appropriate for quantifying bioaccumulation of trace metals by earthworms? The answers to these four questions are needed to coalesce conflicting findings of earthworm metal bioaccumulation to move towards the generation of universally applicable relationships between soil and earthworm trace metal concentrations.

2. Methods

2.1 Search Protocol

Our meta-analysis utilized the rich-body of ISI-Web of Science listed literature concerning trace metals in soils and their uptake by earthworms, ranging from laboratory conditions to field experiments. The literature search of peer-reviewed publications published before June 2019 reporting results on bioaccumulation of trace metals by earthworms was performed using the ISI-Web of Science research database (e.g. Van Groenigen et al., 2014, 2019). We used the following search term:

((TS=(earthworm$ AND soil AND (trace metal$ OR heavy metal$ OR micronutrient$ OR potentially toxic element$ OR metal$) AND (*bioaccum* OR biocon*) NOT vermicompost*))).

The search yielded 267 studies that contained the desired search terms in their titles, abstracts, keywords, and KeyWords Plus, which are words and phrases frequently used in the references of an article. Studies not written in English were not included in these results.

2.2 Study selection

Studies were screened by carefully reading all 267 abstracts to determine suitability of the query search results. Studies that included an experimental treatment that may influence bioaccumulation rate such as soil sterilization, fungi or bacterial amendments, addition of pesticides were not included. Studies that focused on non-mineral soil media were not included such as sewage sludges, organic horizons, and subaqueous soils. Soils that utilized metal treatments, such as sludges, metal salts, or contaminated soils from other areas were included in our study. A total of 119 full-texts were acquired for further inspection. Studies that were unable to be used in our study had one or more of the following issues: failure to report data in accessible format (e.g. data across treatments or sampling sites were not reported, or only reporting aggregate data), missing data set (e.g. soil concentrations not reported), failing to mention depuration of earthworms, not reporting concentrations as dry weight. Authors of recent studies (after 2005) focusing on several trace metals were contacted for data sets but all requests were unsuccessful. We excluded 63 studies of the 119 full texts screened and only 56 studies (Supplemental Table 1) met our criteria for use in our meta-analysis. All data are available in supplementary material. Field studies included in our meta-analysis included different ecosystems (forests, grasslands, agroecosystems), several climatic biomes (temperate, continental, tropical and subtropical), and multiple types of experimental designs (indoor and outdoor pot experiments, field plots of contaminated, uncontaminated, urban and preserved ecosystems).

2.3 Data collection and extraction

Important study metadata were collected (Year Published, First Author Last Name, Metal source as described in the study), earthworm information (Earthworm family, genera, species), experimental design (uptake duration, extraction method and instrumental used for trace metal analysis, treatments or site name, and number of replicates) and chemical data (%SOM, pH, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sb, U, Zn soil and earthworm tissue concentrations). Data were extracted from the 56 studies by transcription when presented in tables while data represented graphically was extracted manually using PlotDigitizer Version 2.6.6, released April 27th, 2014 (http://plotdigitizer.sourceforge.net). The 56 studies contained 951 soil-earthworm trace metal concentration paired data sets, with 3513 data points across 11 trace metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sb, U, Zn).

2.4 Statistical Analyses

Descriptive statistics were calculated using MATLAB (Mathworks, Natick, MA, USA) For the figures and in text data, average values are given ± 1 standard error of the mean. BAF were calculated as the ratio of earthworm to soil trace metal concentrations using values obtained from each study without log-transformation. Descriptive statistics for the pooled soil, earthworm, and BAFs are given in Table 1. Power regressions, also commonly referred to as log-linear regressions, were used to quantify the relationship between soil concentrations and earthworm tissue concentrations in MATLAB. Linear regressions were not used because of their sensitivity to higher concentration values over lower concentration values. Soil concentrations, earthworm concentrations, and bioaccumulation factors were log-transformed and analyzed for normality using the Lilliefors test (Lilliefors, 1967). Earthworm trace metal concentrations and bioaccumulation values were compared across earthworm genera, metal sources, and experimental design conditions, where applicable using generalized linear mixed-effect models (GLMMs) in MATLAB.

For the GLMMs, earthworm metal concentrations and soil concentrations were log-transformed, continuous variables (soil trace metal concentrations, %SOM, and pH) were treated as fixed effects and categorical (ecophysiological group, earthworm genera, duration of metal uptake and source of trace metals) as random effects. The GLMM model consisted of Normal Distribution and Maximum pseudo likelihood fit method. This GLMM configuration was selected based upon the paired data distribution, residual plots, and Akaike information criterion (AIC) values. Results for the GLMM analyses are given in Table 2. Interactions among the experimental design variables (ecophysiological group, earthworm genera, duration of metal uptake and source of trace metals) were explored for log-transformed earthworm tissue concentrations for metals with the most robust data sets (Cd n = 579, Cu n = 608, Pb n =593, and Zn n = 601). To test for data set biases in earthworm tissue concentrations among earthworm genera, uptake duration groups, and ecophysiological groups, an N-Way ANOVA with post-hoc t-tests were performed using MATLAB.

3. Results and Discussion

3.1.1 Earthworm trace metal concentrations

Our meta-analysis of 56 studies shows that earthworms are able to bioaccumulate potentially hazardous concentrations of many toxic metals. Median earthworm tissue concentrations of Hg, Pb, and Cd were above concentrations found to be hazardous for consumption of rodents and fowlby the United States National Research Council (Table 1) (NRC, 2006). Moreover, mean and median earthworm tissue concentrations show bioaccumulation of Zn, Ni, and As near levels that may be hazardous to small mammals and avian (Table 1). The extent to which earthworms bioaccumulate trace metals is influenced both by the regulation of internal tissue concentrations by earthworms (Spurgeon and Hopkin 1999; Karaca et al., 2010; Natal-da-Luz et al., 2011) and by the chemical bioavailability of the trace metals in the soil they inhabit (Bradham et al 2006; Natal-da-Luz et al., 2011).

Most of the 56 studies included in our meta-analysis focused on contaminated soils. From Table 1 however, we observe that median soil concentrations for Hg, Cd, Cu, Zn, Mn, Cr, Ni, As, and U are not greater than background concentrations (Table 1). Most median soil concentrations fell within the range of typical soil concentrations for trace metals as reported by Adriano (2001) and Kabata-Pendias and Mukherjee (2007). However, arithmetic mean and third quartile (Q3) of soil concentrations were substantially elevated above background soil concentrations for Hg, Pb, Cd, Cu, Zn, and Sb (Table 1). Many of the trace metal concentrations in samples were near background due to their role as a control soil in experiments that also included contaminated soils, or where soil was collected from non-point source contaminated sites. The elevated trace metal concentrations are from sites that have historical legacies of smelting (e.g. Nannoni et al., 2001; Zhang et al., 2009), agricultural soils following application of biosolids and sewage (e.g. Liu et al., 2005; Centofanti et al., 2016), or former mining operations (e.g. Morgan and Morgan, 1990; Sizmur et al., 2011; Wang et al., 2018). Elevated concentrations in soils were also observed in soils artificially amended in the laboratory using metal salts (e.g. Dang et al., 2015). Mean Hg, Pb, Sb, and U soil concentrations were skewed far above the interquartile range (Table 1), indicating some experimental designs utilized concentrations that far exceed values commonly found in the environment.

To explore the role of soil concentrations on earthworm trace metal uptake, we used power also referred to as log-linear regressions, as opposed to linear regressions, to avoid bias towards higher concentrations with larger numbers. Power regressions showed that the soil concentrations strongly predict uptake of Hg and Cd in earthworm tissues ($p < 0.01$, $R^2 > 0.35$, Supplemental Figure 1). Soil concentrations of the other metals, Cu, Cr, Pb, Zn, Ni, Mn, and As, as well as Sb and U not shown in Supplemental Figure 1, did not predict earthworm uptake, explaining less than 20% of the variation in tissue concentrations ($R^2 < 0.20$; $p > 0.05$; Supplemental Figure 1). These results agree with previous studies that found Hg and Cd soil concentrations drive uptake across several earthworm species (e.g. Richardson et al., 2015; Da Silva et al., 2016; González-Alcaraz et al., 2018; Wang et al,. 2019). Moreover, the results agree with previous studies that found soil concentrations did not drive Cu, Zn, or Pb earthworm tissue concentrations (e.g. Nahmani et al., 2007; Wang et al., 2012; Richardson et al., 2015; González-Alcaraz et al., 2018).

We further investigated the influence of soil concentrations on earthworm tissues concentrations using generalized linear mixed effect models (GLMM). The model was structured as [Earthworm] = 1 + Ecophysiological Group + Genera + [Soil] + Metal Source + Uptake duration + pH + organic matter, where all variables were categorical except for soil concentrations, pH, and organic matter. GLMM results show that when source of metal, duration of exposure to metals, and soil parameters are taken into account, soil concentrations were significantly correlated with earthworm tissue concentrations for Hg, Cd, Cu, Zn, As, Sb, and U (Table 2, $p < 0.05$). From these results, we hypothesised that the contrasting findings of these studies were due to differences in the earthworm species adopted and other differences in experimental design across the 56 studies. These issues are further explored in the following sections.

3.1.2 Earthworm trace metal concentrations across ecophysiological groups and genera

Earthworm tissue concentrations were significantly different between ecophysiological groups for Hg, Pb, Cd, Zn, Sb, and U using GLMM ($p < 0.05$) but not for Cu, Mn, and Cr. Epigeic earthworms had significantly higher Hg, As, and Sb tissue concentrations than the other ecophysiological groups (Figure 1). Epi-endogeic earthworms had similar concentrations as endogeic earthworms for Pb, Cd, Cu, Zn, Mn, Cr, Ni, As, and U (Figure 1), but had significantly lower Hg concentrations than endogeic earthworms. These results suggest that no ecophysiological group consistently achieves higher or lower trace metals concentrations. Furthermore, the uptake of several metals were not influenced by ecophysiological group at all, hinting that choice of food (i.e. mineral soil vs litter) or dermal contact does not affect their uptake. Lastly, differences in trace metal tissue concentrations between ecophysiological groups may be influenced by additional variables not considered such as variations between species within a group.

Our GLMM analysis indicates that earthworm tissue concentrations varied among earthworm genera for most metals (Hg, Pb, Cd, Cu, Zn, Mn, Sb, U) (Table 2). When focusing on specific earthworm genera, our analysis only compared genera where $N > 10$ for at least five of the metals analyzed in this study. Thus, comparisons for *Diplocardia, Drawidia, Pontoscolex, Octolaision* and *Sparganophilus* were not included in this study due to small sample sizes. There does not appear to be any genera most adept at bioaccumulating all metals, as differences among genera were metal specific. For example; *Eisenia* had significantly higher As and Hg concentrations than all other genera; *Aporrectodea, Dendrodrilus, Eisenoides,* and *Lumbricus* had the highest Pb concentrations, and; *Dendrodrilus* had the highest Cd concentrations ($p < 0.05$; Supplemental Table 3). Moreover, several of the trace metals (e.g. Cu, Zn, and U) for which tissue concentrations were significantly affected by genera in the GLMM (Table 2) had similar tissue concentrations across most genera ($p > 0.10$; Supplemental Table 3). There are important within-genus differences to take into consideration. First, earthworms within the same genus can have very different feeding and burrowing habits (e.g. anecic *Lumbricus terrestris* and epi-endogeic *Lumbricus rubellus*). Second, earthworms within the same genera may inhabit different soils affecting their exposure to trace metal concentrations. Lastly, physiological differences such as their length and surface area of folds within their intestines and excretion capabilities influence metal concentrations in their tissues (Morgan and Morgan, 1990; Morgan and Morgan, 1992; Spurgeon and Hopkins 1999).

3.2.1 Earthworm bioaccumulation factors

Our meta-analysis of 56 studies shows that earthworms consistently bioaccumulated Hg, Cd, and Zn on the basis of Q1 and Median BAFs > 1.0 (Table 1) and power regressions (Supplemental Figure 1). Earthworms were able to bioaccumulate Pb, Cu, Cr, Ni, Sb, As, and U only under certain circumstances, on the basis of Q3 BAFs > 1.0 (Table 1) and power regressions (Supplemental Figure 1). The limited bioaccumulation of Pb, Cu, Cr, Ni, Sb, and U, were likely driven by two specific conditions: highly elevated soil concentrations with reduced uptake tissue concentrations due to saturation and very low soil concentrations with low earthworm uptake causing BAFs to not exceed 1.0. When examining soil concentrations and BAFs in XY space in Supplemental Figure 2, it is clear that As, Cd, Cr, Cu, Hg, Mn, Ni, and Zn have significantly higher BAF when soil concentrations are low ($p < 0.05$, R^2 ranged between 0.14 and 0.78). Lead BAF was not significantly affected by soil concentration ($p > 0.10$, $R^2 = 0.00$). One mechanism for decreasing BAFs with increasing soil concentration is mistaken scavenging as limiting essential elements (such as Hg for Se in Richardson et al., 2015) and increased regulation and excretion at elevated concentrations to maintain homeostasis (such as Mn for Ca in Morgan et al 2007).

We investigated the importance of earthworm type (ecophysiological group, genera) and soil properties (soil extraction methods, soil pH, SOM), accounting for differences in experimental design (source of metals, uptake duration) using generalized linear mixed-effect models (GLMMs). The GLMM for BAF was structured as [Earthworm] = 1 + Ecophysiological Group + Genera + Soil Extraction Method + Metal Source + Uptake duration + pH + organic matter, where all variables were categorical except for pH and organic matter. Since soil concentrations are used to calculated BAF, they cannot be added to the model. The BAF GLMM results are given in Table 3 and described and interpreted in the following sections.

3.2.2 Earthworm bioaccumulation factor across genera and ecophysiological groups

GLMMs revealed that earthworm genera was a significant factor influencing BAF for all trace metals. Our BAFs in Supplemental Table 3 show some genera bioaccumulated metals at higher rates than others but no specific genus consistently bioaccumulated the highest concentration of all trace metals. For example, Lumbricidae genera (*Allobophora*, *Aporrectodea*, *Dendrobaena,* and *Dendrodrilus*) all bioaccumulated Pb, Cd, Cu, Zn, and Ni at greater rates than Megascoelidae genera *Metaphire* and *Pheretima* group (Supplemental Table 3, $p < 0.05$). Similar to the GLMMs for earthworm tissue concentrations, our analysis only compared genera where N > 10 for at least five of the metals analyzed in this study, thus, *Diplocardia*, *Drawidia, Pontoscolex*, *Octolaision* and *Sparganophilus* were not included. Moreover, comparisons between genera are limited as earthworms within the same genus can have different feeding and burrowing habits and may also be influenced by their preferred soil physiochemical properties. In addition, there are physiological differences between earthworms to consider. For example, the substantially reduced calciferous glands of *Megascolecidae* compared to earthworms of Lumbricidae (both *Aporrectodea* and *Lumbricus*) may influence the assimilation and bioaccumulation of trace metals.

Earthworm ecophysiological groups had different BAFs for most metals (Table 3). Endogeic earthworms had significantly higher BAFs for Pb, Cd, Cr, Sb than all other groups from GLMMs ($p < 0.05$; Figure 1). Further, epigeic earthworms had significantly higher BAFs for Hg, Cu, Ni, and As than all other ecophysiological groups from GLMMs ($p < 0.05$; Figure 1). Lastly, anecic earthworms had the lowest BAFs for Hg, Pb, Cd, Ni, and Sb from GLMMs ($p < 0.05$). BAFs can be high for epigeic earthworms due to high metal concentrations in the organic rich soils they inhabit at high densities and endogeic earthworms can live in low organic matter soil found in urban areas and point source polluted sites such as smelters (e.g. Morgan and Morgan, 1990). Anecic earthworms consume fresh plant litter that typically have lower trace metal concentrations than the partially decomposed organic matter consumed by epigeic and endogeic earthworms (Bohlen et al., 2004; Karaca et al,.2010; Richardson et al., 2015). Moreover, anecic earthworms can perform 'external' rumen digestive actions, in which they re-ingest previously digested soils to consume fungal grazers and colonizing microbial communities (Lavelle et al 1994). Epi-endogeic earthworms did not have BAFs resembling endogeic or epigeic earthworms, highlighting their adaptive feeding behavior (Figure 1). Additional studies are required to investigate comparability across metal concentrations, the earthworm diets, and field versus laboratory conditions, all of which can influence trace metal bioaccumulation and retention in their tissues.

3.3.1 Experimental design – Source of metals

The bioavailability of metals is strongly dependent on its phase in soil, as metals present in native silicates or forged-alloyed metals by humans are generally unavailable for immediate uptake by earthworms while exchangeable or dissolved forms are readily available for uptake.

Frequently studies focus on one type of metal source and have not compared how the source of a metal affects the interpretation of uptake and bioaccumulation results. Using GLMMs, we found that the source of metals significantly impacted earthworm tissue concentrations for Hg, Pb, Cd, Cu, Zn, Cr, Ni, and As (Table 3). We further examined this effect in Figure 2 to determine if there were any trends among types of metal sources. Our results show that earthworms inhabiting soils affected by mining activities, smelting, laboratory spiking, and non-point source pollution (e.g. urban soils) had higher tissue concentrations of Hg, Cd, Cu, Cr, Ni, and As than earthworms exposed to background soil concentrations found in pristine environments (Figure 2). Further, we observed that earthworms in agricultural soils exhibited tissue concentrations similar to, or below, the tissue concentrations of earthworms exposed to background soil concentrations for Pb, Zn, and Mn (Figure 2). We therefore conclude that the source of metal increased uptake of trace metals by earthworms rather than not simply elevated concentrations pollution consistently results in.

GLMMs showed that the source of metal can significantly influence BAFs, which may be the result of experimental design. Experiments using laboratory spiking methods, where a metal salt is added to a soil, produced BAFs that were significantly higher than background BAFs for Hg, Cu, Ni, and As (Figure 2). However, this effect was not consistent since laboratory spiking generated a very low Pb BAFs and did not affect Cd, Zn, Mn, and Cr BAFs, compared to background BAFs (Figure 2). Mining and smelting activities did not produce significantly higher BAFs for Pb, Cd, Zn, Mn, and Ni when compared to background BAFs but did generate inconsistent positive and negative effects on BAFs for As, Cr, Cu, Hg, and U (Figure 2). We hypothesise that soil properties and concentration of laboratory spiking method can generate artefacts for testing bioaccumulation due to differences in complexation, sorption, and precipitation (Kumpiene et al 2008). The high solubility of trace metals applied by laboratory spiking can result in higher dissolved concentrations in the soils to which the earthworms are exposed (Nahmani et al., 2007), which may be unrealistic when compared to natural systems that have had longer for the soil to 'age' and the dissolved concentration is allowed to come into equilibrium with the adsorbed or precipitated phase.

Soil properties were important variables influencing BAFs for some metals. Da Silva et al (2016) spiked low pH soils (pH 4) with high concentrations of Hg, creating a large bioavailable Hg pool and high BAFs while Wijayawardena et al., (2017) spiked high pH soils (pH 5 – 8.5) with Pb, creating a large insoluble, unavailable Pb reservoir with low earthworm BAFs when assessed for total soil Pb. Soil concentrations in highly contaminated systems may be elevated to the point that where BAFs are low even though tissue accumulation is high. One example is soil near mining and smelting operations. In these systems, high concentrations in the soil drive high accumulation in earthworm tissues but the BAF remains low because it is defined as the ratio of tissue to soil concentrations. Non-point source pollution did not have significantly different BAFs than background BAFs for most metals: As, Cd, Cu, Cr, Hg, Pb, Mn, and Ni, and Zn, (Figure 2). These results suggest that using a source of metal contamination that best mimics natural systems can recreate natural bioaccumulation pathways of metal uptake while still generating elevated earthworm tissue metal concentrations. Laboratory spiking of soils with trace metals or using point source polluted sites from mining or smelting has the potential to generate experimental artefacts when findings are applied to non-point source polluted sites (e.g. degraded areas or urban areas) and limit broad applicability of results.

3.3.2 Experimental design – Exposure duration to metals

The duration that earthworms are exposed to a soil can influence the bioaccumulation of metals, as earthworm require time to attenuate to soil metal concentrations through soil ingestion (see Spurgeon and Hopkin 1999) and passive diffusion across their skin (Vijver et al 2003). Our GLMM analysis shows earthworm tissue concentrations for Hg, Pb, Cd, Cu, Zn, and As were significantly influenced by the duration of exposure to the soils (Table 2). Although one would expect the longest duration to cause the greatest uptake of metals, this was not always the case. Longer exposure durations to Cd and Zn produced the highest earthworm tissue concentrations (Figure 3), but short and medium duration experiments generated the highest concentrations of Hg, As, and Sb (Figure 3).

Our GLMM analysis showed BAFs for Hg, Pb, Cd, Cu, Zn, and As were significantly influenced by the duration of exposure to the soils (Table 3). Short duration experiments (< 2 weeks) generated the lowest BAFs for Hg, Pb, Cd, Cu, Zn and Sb compared to entire life durations (Figure 3). Medium duration experiments (3 to 6 weeks) generated low BAFs for Pb, Cd, and Sb and high BAFs for Hg, Cr, Ni, and As compared to entire life durations (Figure 3). Similarly, long duration experiments (6 to 20 weeks) were more closely aligned with entire life studies for some metals (Cd, Cu, Zn, Mn) but also generated metals with significantly higher (Cr, Ni) or lower (Pb, As) BAFs compared to entire life studies (Figure 3).

These results highlight that duration of experiments can also limit interpretations from laboratory-based experiments to field experiments. As with comparisons of earthworm tissues among experiment durations, several factors regarding duration of exposure could be responsible for the effect. First, short experiments can use concentrations that negatively impact their health and alters physiology and behavior, or are lethal but their short duration allows for survival. Second, earthworms may be unable to attenuate to a dynamic equilibrium of tissue trace metal concentrations (particularly for essential elements) within the experimental duration (Spurgeon and Hopkins 1999). Lastly, there may be covariance with the metal source as short duration experiments with high soil metal concentrations typically use soils spiked with metal salts which are highly bioavailable (Nahmani et al, 2007). Thus, experiment duration may be an important variable or covary with other variables and additional field-based studies are needed uptake and bioaccumulation under natural conditions.

3.3.3 Experimental design – Extraction method impact on BAFs

There are dozens of standardized extraction and digestion methods to assess trace metals in soils with varying purposes, ranging from assessing mobility, exchangeability, inorganic sorption, organic complexation, precipitation within secondary oxides, silicate forms, and total concentrations (Rao et al., 2008). The choice of extraction procedure may meet specific research aims for evaluating soil, but may affect comparability when calculating BAFs. Our GLMM found that soil extraction method significantly biased BAFs for most metals: Pb, Cd, Cu, Zn, Cr, Ni, and As (Table 3).

In Supplemental Figure 3, we compared BAFs calculated from five categories of extraction methods: water soluble being the least exhaustive, exchangeable focusing on cation exchangeable metals using a salt (e.g. $CaCl_2$ or $MgCl_2$), extractable using an organic ligand (e.g. EDTA or DTPA) or weak acid (dilute nitric acid or acetic acid), pseudototal digestion (e.g. concentrated HNO_3, HCl, H_2SO_4 or some combination), and total digestions (HF, $HClO_4$, H_3PO_4). Our analysis shows that pseudototal and total digestions consistently produced BAFs that were similar for all metals (Supplemental Figure 3). Using exchangeable or extractable soil concentrations consistently generated higher BAFs than pseudototal or total digestion methods (Supplemental Figure 3). However, BAFs measured using water soluble phases produced BAFs similar to pseudototal or total digestion for some metals (e.g. Cd, Cu, Ni) but also generated significantly higher BAFs for other metals (Zn and As).

The impact of the soil extraction method on BAFs has two important ramifications for considering if metals are bioaccumulated and to what extent. First, using BAFs relies on the assumption that >1.0 means metals are actively bioaccumulated by earthworm physiologically but this analysis shows extraction method can affect these results. For example, Cu and Ni BAFs measured with pseudototal and total digestions are <1.0, suggesting they are not actively bioaccumulated. However, if exchangeable and extractable concentrations are used to calculate BAFs for Cu and Ni, then BAFs are >1.0 and they are considered actively bioaccumulated. We recommend using BAFs for pseudototal and total digestions, as other extraction procedures may overestimate BAFs through underestimating soil metal concentrations. Second, if other soil extraction methods are desired, the assumption of 1.0 being an inflection point of bioaccumulation may need to be reconsidered and a new point dependent on the soil extraction method would be warranted. However, we argue that authors should avoid this later framework for consistency in the literature.

3.3.4 Interactions among experimental design and data set biases

Our N-Way ANOVA analysis found significant interactions among earthworm genera, uptake duration, and ecophysiological groups for Cd, Cu, Pb, and Zn (Supplemental Table 2). As a prime example, litter-feeding and dwelling earthworms of the genus *Eisenia fetida* were consistently used in shorter duration laboratory experiments than mineral soil dwelling, earthworms genera conducted for their entire lifetimes under field conditions. This is simply due to the fact that *Eisenia fetida* are a preferred model soil dwelling laboratory organism due to their short life cycle, maturation in ~50 days, ease of care on organic wastes, and ability to reproduce and live in high densities (OECD 1984).

In spite of our efforts to include a diverse array of studies on bioaccumulation, it is important to note key limitations and biases in our data set. First, trace metal data were primarily Cd, Cu, Pb and Zn data ($n > 500$), while metals such as Cr, Hg, Mn, Ni, Sb, U were reported less often ($n < 200$). Second, data from agricultural areas and non-point source polluted sites are underreported ($n < 0$ to 51) compared to areas near mining and smelting activities (n = 70 to 150). Third, Lumbricidae were overrepresented (mean across trace metals n = 41%) compared to Megascolecidae (mean n = 10%) and Glossoscolecidae (mean n = 6%). Fourth, anecic earthworms (mean across trace metals n = 10%) were understudied compared to epigeic (mean n = 44%), endogeic (mean n = 23%), epi-endogeic (mean n = 23%) earthworms. Lastly, our study did not utilize the breadth of studies examining toxicokinetics, commonly due to additional treatments affecting uptake and excretion rates. Thus, our study primarily utilized organisms that spent their entire life cycle in the soil (mean across trace metals n = 73%) as opposed to shorter exposure durations.

4. Conclusions

The uptake and bioaccumulation of trace metals is important for ecotoxicological research to ensure earthworm predators are not at risk of toxicity and an underappreciated aspect of soil biogeochemistry. Our study demonstrated that specific metals, such as Hg, Cd, and Zn are taken up and bioaccumulated across earthworm genera. Other metals, such as Pb, Cu, Ni, As, can also be taken up and bioaccumulated under certain conditions. Traditionally, we consider the primary driver of trace metals in earthworm tissues to be their respective soil concentrations.

However, many other factors play a role in uptake, particularly for metals where earthworm and soil concentrations were poorly correlated: Pb, Cu, Zn, Mn, Cr, Ni, As, Sb, and U. These additional factors can be environmental conditions, which include, but are not limited to, genus of earthworm, ecophysiological group, soil pH, and organic matter content. Moreover, anthropogenic activities can also control the uptake and bioaccumulation of trace metals through different trace metal sources (e.g. non-point source pollution, smelting, mining). Unfortunately, the manner by which we study uptake and bioaccumulation of trace metals can generate artefacts that limit generalizability of results from many studies. Experimental design limitations include the spiking soils with substantially elevated concentrations of metals in the laboratory, reduction of the duration of exposure before full effects may be realized, and underestimating total metal concentrations with weak extraction procedures.

5. Research Needs

5.1 Reporting full trace metal sets

Many studies only report values for a limited set of trace metals and determining co-variance and element competition has remained largely unexplored. When possible, reporting full sets of trace metals analyzed with appropriate QA/QC should be a standard. Measurement of a consistent suite of metals aids researchers interested in other trace metals and also provides insights into whether metals are co-varying or co-bioaccumulated by earthworms. We recommend that researchers utilizing Atomic Absorption Spectroscopy, Inductively Coupled Plasma Optical or Atomic Emission Spectrometry, and Inductively Coupled Plasma Mass Spectrometry measure As, Cd, Cu, Pb, Ni, and Zn as primary suite of common inorganic soil contaminants. A secondary suite consisting of Co, Cr, Sb, Sn, U, and W are proposed as a suite of emerging and site-specific pollutants that may be elevated in earthworms, but the literature severely lacks data on these metals. The measurement of Hg should only be done with either a direct mercury analyzer or an established ICP-MS protocol. Lastly, data should be published in accessible formats. Tables with metal concentrations for each treatment, each site, or lowest applicable treatment unit so data can be further interpreted. Cumulative figures and in-text reporting are not recommended for promoting accessible data. With widely available supplemental data submissions with publishers and data repositories, researchers must consider making their data available for future studies to build upon.

5.2 Exploring earthworms beyond E. fetida, L. terrestris, and L. rubellus

The abundance of studies on *L. terrestris* and *E. fetida* are not a surprise as they have been considered model organisms for laboratory study. In our study, *E. fetida* (N = 141/951), *L. terrestris* (N = 67/951), and *L. rubellus* (N = 104/951) were the three most commonly studied species of earthworms, constituting 33% of the earthworms studied. While this is advantageous for reproducibility when studying molecular scale processes, physiological responses, and genetic processes, it severely limits application to field studies where hundreds of species are understudied. Moreover, the focus on *E. fetida* is problematic as it is a small, organic-rich soil dependent earthworm, most commonly studied under laboratory conditions, and data focused on this earthworm skew results towards their preferred type of soil environment. Our GLMM results show that pH and organic matter can significantly impact earthworm tissue concentrations and bioaccumulation of trace metals. Thus, additional studies on uptake and bioaccumulation of endogeic and epi-endogeic earthworms are needed and should consider being conducted at the earthworm community-level. Moreover, further studies on Asiatic and American earthworms of the families *Megascolecidae*, *Acanthodrilidae*, *Moniligastridae*, and *Glossoscolecidae* are required to further our understanding.

5.3 Earthworm field studies at larger scales

Most field studies have focused on limited point-source polluted sites. However, this causes a lack of field scale studies investigating soils at the ecosystem level and their influence on earthworm uptake and bioaccumulation of trace metals. More regional to continental scale studies are needed to accurately capture the influence of soil properties (e.g. pH, SOM, texture, structure) and environmental parameters (e.g. soil moisture, temperature) on metal uptake and bioaccumulation. In addition, changes to metal cycling in the environment can influence many other properties important at the global scale (e.g. organo-metalloid disruption releasing DOC, leaching of nutrients decreasing plant growth).

5.4 Earthworms in agricultural settings

Another effect of focusing on laboratory soils with amended trace metal concentrations, is a lack of data on background metal concentrations across earthworm genera. Field and laboratory studies are needed to determine background, natural, or uncontaminated concentration data for earthworms. Due to the limited background data, it is difficult to assess if earthworms are exhibiting contaminated or polluted trace metal concentrations or if these are differences due to their physiology.

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Table 1 Soil and earthworm trace metal concentrations and calculated bioaccumulation factors across all 56 studies.

		As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Sb	U	Zn
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Soil concentrations	Q1	1	0.3	10	14	0.06	389	5	14	0.1	0.11	53
	Median	3	1.7	19	25	0.09	912	9	63	3.1	0.27	129
	Mean	78	12.4	132	117	24.76	811	170	613	23.8	0.91	512
	Q3	22	5.8	57	77	0.39	1214	19	236	9.0	0.53	384
Background soil concentrations†		0.1–55	0.01–2	10–150	4–70	0.03–0.2	2-5000	5–200	5–40	0.1–2.2	0.1–102	10-300
Earthworm tissue concentrations	Q1	2	4	4	11	0.2	63	2	10	0.20	0.16	139
	Median	4	15	11	18	0.6	153	5	32	0.39	0.31	330
	Mean	53	44	22	34	44.7	202	12	181	4.25	0.42	545
	Q3	14	52	27	39	1.7	291	12	109	1.17	0.57	660
Concentrations hazardous to rodents and fowl‡		30	10	100	250	0.2	2000	50	10	-	100	500
		mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}	mg kg^{-1} /mg kg^{-1}

Bioaccumulation factor	Q1	0.20	4.22	0.11	0.30	1.50	0.11	0.24	0.11	0.06	0.56	0.96
	Median	1.11	9.49	0.31	0.67	3.44	0.25	0.50	0.40	0.14	1.25	2.09
	Mean	140.4	25.4	67.3	12.1	13.4	0.64	2.40	6.36	21.24	1.48	9.83
	Q3	5.17	21.3	1.41	1.45	9.37	0.57	1.25	1.55	4.94	1.96	6.02
Observations	N	330	579	95	608	199	142	164	593	90	111	601

†Background soil concentrations are from reported values from Adriano (2001), Smith et al., (2014) and Kabata-pendias and Mukherjee (2007).
‡Concentrations from Mineral Tolerance of Animals: 2005 by the United States National Research Council, NRC(2006).

Table 2 Model output p-values from generalize linear mixed effect models for earthworm trace metal tissue concentrations across 56 aggregated studies for random and fixed variables. (+) indicates a positive effect and (-) indicates a negative effect of a variable. Akaike information criterion (AIC) values for selecting each model are also given.

Metal	N	Ecophysiological Group	Earthworm Genera	Soil concentration	Metal Source	Uptake duration	Soil pH	Soil organic matter	Model AIC
As	329	n.s.	<0.01	(+)<0.01	<0.01	(+)<0.01	n.s.	(+)<0.01	338
Cd	580	<0.01	<0.01	(+)<0.01	<0.01	(+)<0.01	n.s.	(-)<0.01	379

Cr	96	n.s.	<0.01	n.s.	<0.01	n.s.	(+)<0.01	n.s.	65
Cu	608	n.s.	<0.01	(+)<0.01	<0.01	(+)<0.01	n.s.	(-)<0.01	295
Hg	200	<0.01	<0.01	(+)<0.01	<0.01	(+)<0.01	n.s.	(-)<0.01	175
Mn	143	n.s.	<0.01	n.s.	n.s.	n.s.	(+)<0.01	n.s.	102
Ni	165	n.s.	<0.01	n.s.	<0.01	n.s.	(+)<0.01	n.s.	117
Pb	593	<0.01	<0.01	n.s.	<0.01	(+)<0.01	n.s.	(+)<0.01	988
Sb	90	<0.01	<0.01	(+)<0.01	n.s.	n.s.	n.s.	n.s.	48
U	112	<0.01	<0.01	(+)<0.01	n.s.	n.s.	(-)<0.01	n.s.	49
Zn	601	<0.01	<0.01	(+)<0.01	<0.01	(+)<0.01	n.s.	n.s.	317

Table 3 Model output p-values from generalize linear mixed effect models for earthworm BAF values across the 56 aggregated studies for random and fixed variables. (+) indicates a positive effect and (-) indicates a negative effect of a variable. Akaike information criterion (AIC) values for selecting each model are also given.

Metal	N	Ecophysiological Group	Earthworm Genera	Soil Extraction Method	Metal Source	Uptake duration	Soil pH	Soil organic matter	Model AIC

As	329	n.s.	<0.01	<0.01	<0.01	(+)<0.01	n.s.	n.s.	487
Cd	580	<0.01	<0.01	<0.01	<0.01	(+)<0.01	(−)<0.01	(−)<0.01	488
Cr	96	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.	n.s.	95
Cu	608	<0.01	<0.01	<0.01	<0.01	(+)<0.01	n.s.	(−)<0.01	680
Hg	200	<0.01	<0.01	n.s.	<0.01	(+)<0.01	n.s.	n.s.	230
Mn	143	n.s.	<0.01	n.s.	n.s.	n.s.	(+)<0.01	n.s.	129
Ni	165	n.s.	<0.01	<0.01	n.s.	n.s.	n.s.	n.s.	137
Pb	593	<0.01	<0.01	<0.01	<0.01	(+)<0.01	n.s.	n.s.	1077
Sb	90	<0.01	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	76
U	112	<0.01	<0.01	n.s.	n.s.	n.s.	(−)<0.01	n.s.	79
Zn	601	<0.01	<0.01	<0.01	<0.01	(+)<0.01	n.s.	(−)<0.01	699

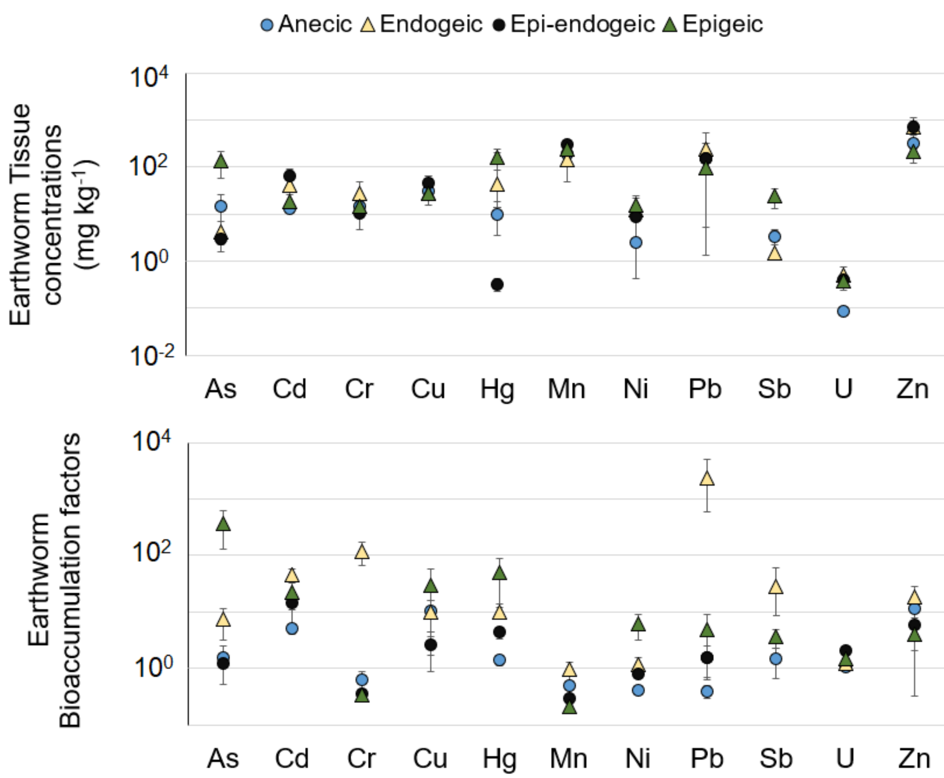

Figure 1 Earthworm trace metal concentrations and bioaccumulation factors (tissue concentrations divided by soil concentrations) across the 57 studies. Error bars are ± 1 standard deviation. N for each plot is given in the supplemental materials.

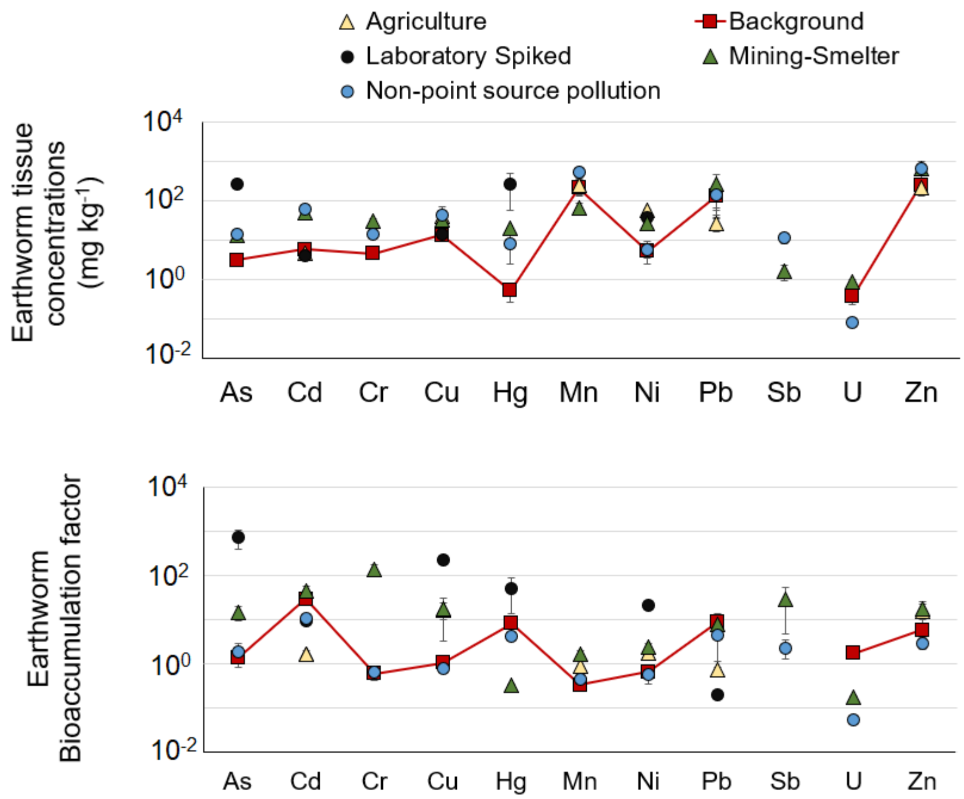

Figure 2 Plots of average earthworm tissue concentrations and bioaccumulation factors examined across types of metal sources for 56 studies. Error bars are ± 1 standard deviation. N for each plot is given in the supplemental materials.

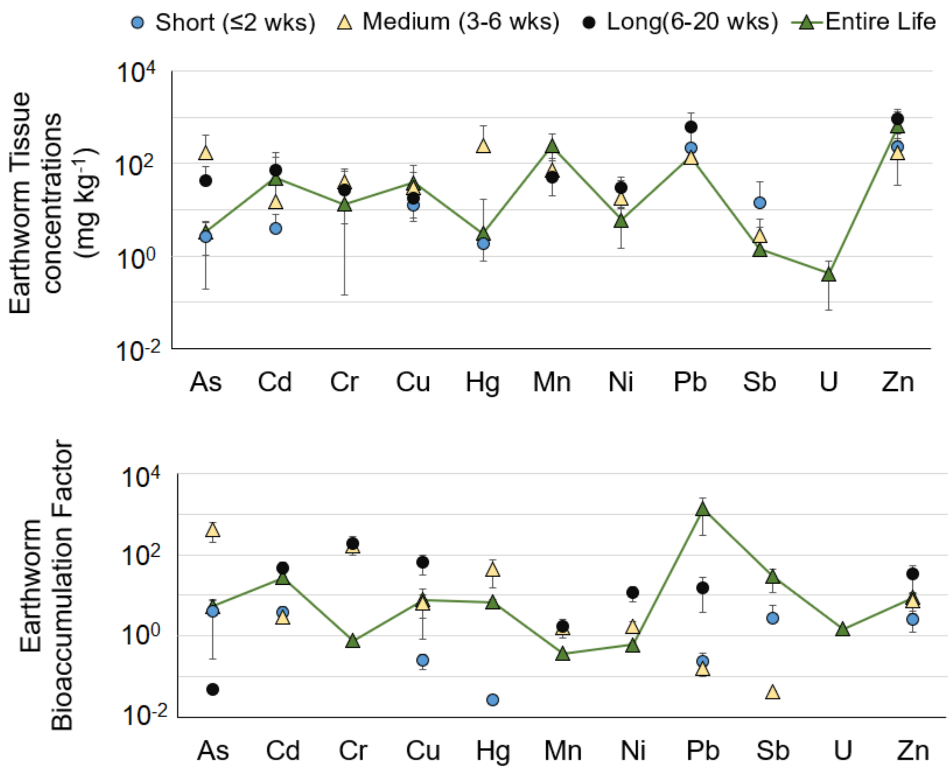

Figure 3 Earthworm trace metal concentrations and bioaccumulation factors (tissue concentrations divided by soil concentrations) examined by duration of metal exposure across the 56 studies. Error bars are ± 1 standard deviation. N for each plot is given in the supplemental materials.