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Sizmur, T. ORCID: https://orcid.org/0000-0001-9835-7195 and Richardson, J. (2020) Earthworms accelerate the biogeochemical cycling of potentially toxic elements: results of a meta-analysis. Soil Biology and Biochemistry, 148. 107865. ISSN 0038-0717 doi: 10.1016/j.soilbio.2020.107865 Available at https://centaur.reading.ac.uk/90825/

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To link to this article DOI: http://dx.doi.org/10.1016/j.soilbio.2020.107865

Publisher: Elsevier

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Earthworms accelerate the biogeochemical cycling of potentially toxic elements: Results of a meta-analysis

Tom Sizmur[1]* and Justin Richardson[2]

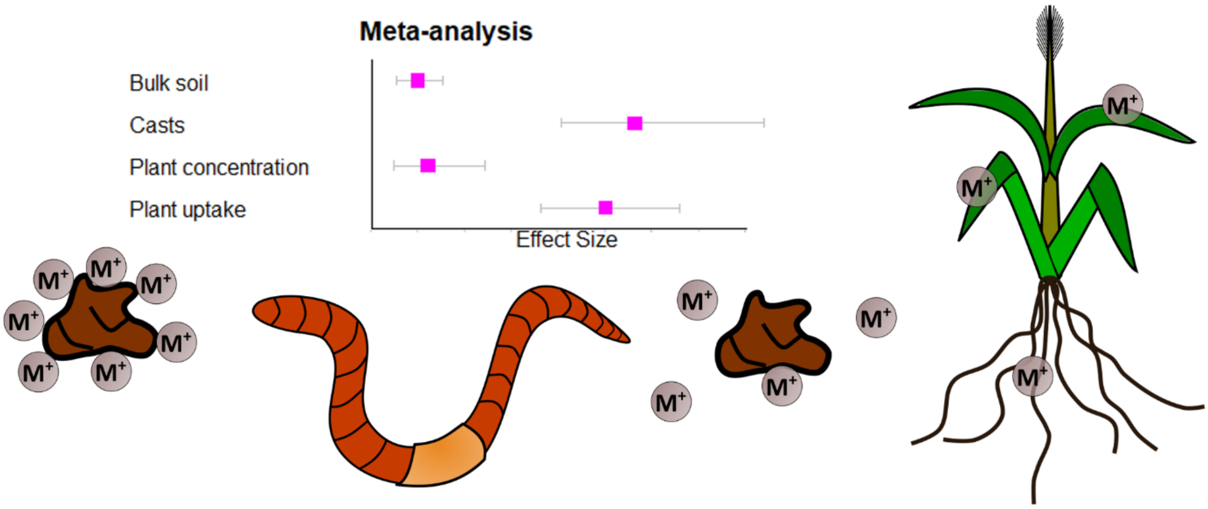
[1] Department of Geography and Environmental Science, Russell Building, University of Reading, Reading, RG6 6DW, UK

[2] Department of Geosciences, Morrill Science Center, University of Massachusetts, Amherst, MA 01003, USA

* Corresponding author: t.sizmur@reading.ac.uk

Abstract

Earthworms are ecosystem engineers, capable of modifying the soil environment they inhabit. Recent evidence indicates that they increase the mobility and availability of potentially toxic elements in soils, but a quantitative synthesis of the evidence required to understand mechanisms and identify soils most susceptible to earthworm-induced potentially toxic element mobilisation is lacking. We undertook a meta-analysis of 42 peer reviewed journal studies, comprising 1185 pairwise comparisons between earthworm-inhabited and earthworm-free soils to quantify the impact of earthworms on potentially toxic element mobility in bulk earthworm-inhabited soil and earthworm casts, and on plant uptake and concentration. We find that endogeic and epigeic earthworms increase the mobility of potentially toxic elements in the bulk soil, and earthworms from all ecological groups mobilise potentially toxic elements during passage of soil through the earthworm gut. We also observe an increase in the concentration and uptake of potentially toxic elements by plants growing on soils inhabited by epigeic (mostly *Eisenia fetida*) earthworms. Earthworms mobilise potentially toxic elements in geogenic soils to a greater extent than anthropogenically contaminated soils. Soils with very low (<2%) soil organic matter content are most susceptible to earthworm-induced potentially toxic element mobilisation. These findings have important implications for the ability of exotic earthworms to alter soil biogeochemical cycles when introduced to new environments. Mixing amendments with contaminated soils with the intention of reducing the mobility of potentially toxic elements may be aided by the activity of earthworms that accelerate the mixing processes. Furthermore, our findings also highlight a promising phenomenon that, if harnessed, may help to alleviate micronutrient deficiencies in degraded soils.



Keywords

Heavy Metals; Availability; Lability; Soil: Pollution; Micronutrients

1. Introduction

Earthworms are considered 'ecosystem engineers' (Lawton, 1994; Jouquet et al., 2006; Lavelle et al., 2006), capable of modifying the physical, chemical, and biological properties of the soils they inhabit (Blouin et al., 2013). As soil passes through the earthworm gut, it is exposed to enzymes that break down organic compounds (Aira et al., 2003; Liebeke et al., 2015; Lipiec et al., 2016) and increase the availability of nutrients (Van Groenigen et al., 2019). Earthworms also release mucus and urine into the soil solution (Scheu, 1991; Salmon, 2001), which can alter the relationship between soluble elements and soil surfaces (Sizmur et al., 2010). Epigeic earthworms feed on organic residues in the litter layer and the first few cm of the mineral soil surface. Anecic earthworms draw litter from the soil surface into permanent vertical burrows, and endogeic earthworms ingest mineral soil, creating non-permanent burrows in the soil matrix. The accidental introduction of exotic anecic earthworms to forests in Northeastern United States and the subsequent disappearance of the litter layer and changes to the soil biogeochemistry (Bohlen et al., 2004; Dobson et al., 2017) highlight the important role that these ecosystem engineers play in the biogeochemical cycling of elements in the soil environment (Frelich et al., 2006). In environments in which they are native, earthworms are considered keystone species because of the role that they play in maintaining healthy soil (Fusaro et al., 2018; Stroud, 2019) and providing a food source for other terrestrial organisms at higher tropic levels (Macdonald, 1983; Edwards, 2004). For this reason, earthworms have been afforded positive recognition and were among the first soil organisms to have a standardised soil toxicity test developed (Spurgeon et al., 2003).

Earthworms are relatively tolerant of potentially toxic elements and often found in soils with elevated concentrations (Spurgeon and Hopkin, 1996; Klok et al., 2007; Nahmani et al., 2007; Richardson et al., 2020). Some earthworm populations have adapted mechanisms to tolerate high concentrations of potentially toxic elements (Spurgeon and Hopkin, 2000; Kille et al., 2013). The knowledge that earthworms are able to increase the availability of nutrients after passage through their gut (Van Groenigen et al., 2019), ultimately leading to greater plant growth (Van Groenigen et al., 2014), along with the observation that earthworm mortality in Pb contaminated soil was significantly greater in multiply occupancy test chambers, compared to single occupancy chambers (Currie et al., 2005), led to a narrative review of the literature that concluded that earthworms increase the mobility and availability of potentially toxic elements in soils (Sizmur and Hodson, 2009). Since this narrative review was undertaken, many more experiments have been conducted to examine the impact of earthworms on potentially toxic elements mobility and uptake by plants, yet, no quantitative synthesis of this published data has been undertaken to identify mechanisms and resolve contradictory findings across studies.

Here, we conducted a systematic review and meta-analysis of the peer reviewed literature, following the approach of Thomas et al. (2019) to determine whether earthworms do increase the mobility of potentially toxic elements in soils and their uptake into plant tissues. We tested two central hypotheses: (1) earthworm-inhabited soils or earthworm casts have a greater mobility of potentially toxic elements than earthworm-free soils; (2) plant concentration and uptake of potentially toxic elements is greater from earthworm-inhabited soils than earthworm-free soils. Further, we also aimed to elucidate the mechanisms by which earthworms alter potentially toxic element cycles and identify the types of soils that are particularly susceptible to earthworm-induced potentially toxic element mobilisation. The source of the elements was also important because the extent to which they are readily soluble, organic bound, or precipitated can affect their susceptibility to mobilisation. More specifically, we hypothesized that earthworm ecological group (anecic, epigeic, endogeic), soil physicochemical properties (pH, organic matter content), metal-specific properties (essential vs non-essential), and experimental design (source of contamination, further amendments, soil extraction methodology) would influence the magnitude of earthworm-induced impacts on potentially toxic element mobility and plant uptake.

2. Material and methods

2.1. Literature search and inclusion criteria

Peer-reviewed journal articles were selected for the meta-analysis using the online database ISI Web of Science. To obtain studies on the influence of earthworms on the mobility of potentially toxic elements in soils, we used the search term:

*earthworm$ AND soil AND (trace metal$ OR heavy metal$ OR micronutrient$ OR potentially toxic element$ OR metal$) AND (*availab* OR mobil*) NOT vermicompost**

To obtain studies on the influence of earthworms on the concentration and uptake of potentially toxic elements from soils by plants, we used the search term:

*earthworm$ AND soil AND (trace metal$ OR heavy metal$ OR micronutrient$ OR potentially toxic element$ OR metal$) AND (*uptake* OR growth*) NOT vermicompost**

These searches, conducted on 28th December 2018, yielded 491 and 451 results, respectively 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. The search results were screened by carefully reading all 942 abstracts to identify studies that may contain suitable data. After this initial screen, the full text was obtained for 63 and 43 studies, respectively. A second screen (based on reading the full text) resulted in 40 and 18 studies, respectively that contained suitable data. We were particularly looking for studies that contained data (means, standard deviations and number of replicates) comparing the mobility or plant uptake of Hg, Pb, Cd, Cu, Zn, Mn, Cr, Ni, As, Sb or U in earthworm inhabited soils and earthworm-free soils. Studies were included if they contained primary data from experiments with earthworm-inhabited treatments and control treatments that were kept earthworm-free, but otherwise identical. Studies were included if potentially toxic element mobility was measured by means of a chemical extraction of soil samples or the analysis of soil porewater. Studies were also included if potentially toxic element concentration was measured by analysis of plant tissues with, or without, concurrent measurement of biomass (allowing calculation of uptake by multiplying concentration by biomass).

2.2. Data extraction

The mean, standard deviation and number of replicates were extracted from studies pertaining to the mobility of potentially toxic elements in earthworm inhabited soils, earthworm-free soils, earthworm casts, and the concentration in the tissues of plants grown on either earthworm-inhabited or earthworm-free soils. Mobility was defined as porewater, leachate, or an extraction using a solution containing a salt, chelating compound, weak acid, water, or combination of the above. In studies where a sequential extraction was conducted, only data pertaining to the first extraction was considered (unless concentrations from one or more steps were combined by the author and only presented as a sum of the 'available' fractions). Therefore, the definition of a data point was any value reported in a study that resulted from the mean of two or more replicates, reported alongside a measure of precision (e.g. a standard deviation or standard error). All data points reported in a study that met our inclusion criteria, described in section 2.1, were extracted from every study, often resulting in many data points originating from the same study.

If the error reported in tabulated data (often as +/- the arithmetic mean) was not specified then it was assumed that this was a standard deviation of the mean. Data presented graphically was extracted manually using WebPlotDigitizer Version 4.2. Both mean values and error bars were extracted and error bars were assumed to be standard errors, if not specified. To check the accuracy of the digitisation, data from a figure presented in one of our own studies (Sizmur et al., 2011c), for which we had the raw data used to create the plot, was extracted with a 100.8% recovery (SD = 0.28, n = 10). Standard errors presented in tables or figures were converted to standard deviations, where required.

For each pairwise comparison between earthworm-inhabited and earthworm-free treatments, meta-data were collected pertaining to the publication the data was collected from, the earthworm and plant species, the identity and source of the potentially toxic elements in the soil, any further amendments that were made to the soil, the soil pH, and the soil organic matter (SOM) content. Where soil organic carbon only was presented, this value was converted to SOM by multiplying by 1.667, assuming that SOM is 60% carbon. Where plant concentrations were reported alongside plant biomass, uptake was calculated by multiplying the mean concentration by the mean biomass and the standard deviations of both measurements were pooled using Equation 1.

Equation 1 $$\left(\left(\frac{\sigma_1}{\mu_1}\right)^2 + \left(\frac{\sigma_2}{\mu_2}\right)^2\right)^{\frac{1}{2}} \times (\mu_1 \times \mu_2)$$

Where μ_1 and σ_1 are the mean and standard deviation of the plant concentration, and μ_2 and σ_2 are the mean and standard deviation of the plant biomass

2.3. Calculation of effect size and selection of moderators

For each pairwise comparison of two mean data points, the variance was pooled and Cohen's d calculated as the difference between the two mean data points, divided by the pooled variance, as shown in Equation 2.

Equation 2 $$Cohen's\ d = \frac{\mu_A - \mu_B}{\sigma}$$

Where μ_A is the mean of the earthworm-inhabited treatment, μ_B is the mean of the control (earthworm-free) treatment and σ is the pooled standard deviation of both treatments, defined in Equation 3.

Equation 3 $$\sigma = \sqrt{\frac{\left((n_A - 1) \times \sigma_A^2\right) + \left((n_B - 1) \times \sigma_B^2\right)}{n_A + n_B}}$$

Where n_A and n_B are the number of replicates of the earthworm-inhabited treatment and control (earthworm-free) treatment, respectively, and σ_A^2 and σ_B^2 are the variance of the earthworm-inhabited treatment and control (earthworm-free) treatment, respectively.

Cohen's d is the standardised difference between the two mean values. A positive value of Cohen's d indicates that potentially toxic element mobility or plant concentration or uptake is greater in earthworm-inhabited soils, compared to earthworm-free soils. A Cohen's d of >1 indicates that the difference between the means is greater than one standard deviation. Each value of Cohen's d, resulting from a pairwise comparison of two mean data points constitutes a single observation hereafter. Thus, the results presented are means of multiple observations.

The observations were assigned to four datasets (Table 1) representing the influence of earthworms on potentially toxic element mobility in soil, potentially toxic element mobility in casts, potentially toxic element concentration in plant tissues, and potentially toxic element uptake into plant tissues The observations within each dataset were further assigned to groups to aid interpretation and elucidate mechanisms. These groups were selected to test specific hypotheses and the number and size of groups was selected to ensure, where possible a sufficient number of observations to enable statistical power. The moderators that were used to define the groups were:

i. Earthworm ecological group (anecic, endogeic, epigeic).
ii. Individual potentially toxic elements (As, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) and whether they are essential (Cu, Mn and Zn), or non-essential (As, Cd, Co, Cr, Ni and Pb) for plant growth.
iii. Measurements made on the aboveground or belowground tissues of plants.
iv. The source of potentially toxic elements in soils (mining or smelting, urban or industrial pollution, agricultural amendments, artificial spiking of soil, or whether the soil was geogenic).
v. Any further amendments applied to the soil, alongside earthworm treatments (organic amendments, mineral amendments, the pre-washing of soil with chelating compounds, the inoculation with other biological organisms, and the mixing of contaminated soil with uncontaminated soil).

vi. The type of extraction used to determine metal mobility (water extraction, porewater, or leachate, extractions with chelating compounds, weak acids or salt solutions combined steps of a sequential extraction, or a physiologically-based extraction).

vii. pH of the soil prior to earthworm addition (< 5.5, 5.5-6.5, 6.5-7.5, and >7.5).

viii. SOM content of the soil, prior to earthworm addition (< 2%, 2-5%, 5-10% and > 10%).

2.4. Meta-analysis

Data visualisation and statistical analysis were conducted in R version 3.6.0 (R Core Team, 2019), using R Studio version 1.2.1335 (RStudio Team, 2015). A weighted mixed effects model of the Cohen's d values was fit to each dataset using the *rma.mv* function in the *metafor* package (Viechtbauer, 2010). Heterogeneity (Qe) was estimated using the restricted maximum-likelihood estimator. Each moderator was considered as a fixed effect in the model. The publication that the data was drawn from was used as a random factor in the models since in all cases several data points were drawn from the same publication. An omnibus test of all the moderators (Qm) was conducted to determine whether the moderators listed above significantly explained the variation in the data, followed by Wald-type tests for individual moderators using the *anova.rma* function. Individual groups of data were considered to significantly increase or decrease potentially toxic element mobility, concentration or uptake if their 95% confidence intervals did not overlap zero. Since the data was generally non-parametric, non-parametric bootstrap 95% confidence intervals were computed using the *BootES* package in R on groups of Cohen's d values with $n \geq 20$ observations using 10,000 bootstrap resamples, following Kirby and Gerlanc (2013). For groups where $n < 20$, only the mean Cohen's d values were reported, without confidence intervals. Forest plots were generated using the *forestplot* package in R (Gordon and Lumley, 2019).

Table 1 Publications from which data was drawn from that contributed to the four datasets used in the meta-analysis

Publication	Paper no.	Bulk	Casts	Concentration	Uptake
Wu et al., (2019)	1	X		X	X
Elyamine et al., (2018)	2	X		X	
Dehghanian et al., (2018)	3	X		X	X
Kavehei et al., (2018)	4	X			
Li et al., (2018)	5	X		X	X
Maki et al., (2017)	6	X		X	
Wu et al., (2016)	7	X			
Bityutskii et al., (2016)	8		X	X	X
Lemtiri et al., (2016)	9			X	
Zhang et al., (2016)	10		X		
Leveque et al., (2014)	11	X		X	
Du et al., (2014)	12	X			
Tejada et al., (2013)	13	X			
Tica et al., (2013)	14	X			
Jusselme et al., (2013)	15	X			
Macci et al., (2012)	16	X		X	
Bityutskii et al., (2012)	17	X	X		
Bystrzejewska-Piotrowska et al., (2012)	18	X			
Jusselme et al., (2012)	19			X	X
Alonso-Azcárate et al., (2011)	20		X		
Gomez-Eyles et al., (2011)	21	X			
Beesley and Dickinson, (2011)	22	X			
Ruiz et al., (2011)	23	X		X	
Sizmur et al., (2011a)	24	X			
Sizmur et al., (2011b)	25	X	X		
Sizmur et al., (2011c)	26	X		X	X
Sizmur et al., (2011e)	27	X			
Udovic and Lestan, (2010a)	28	X	X		
Udovic and Lestan, (2010b)	29	X			
Fujii and Kaneko, (2009)	30	X			
Ruiz et al., (2009)	31	X		X	X
Kızılkaya, (2008)	32		X		
Coeurdassier et al., (2007)	33	X			
Udovic et al., (2007)	34		X		
Udovic and Lestan, (2007)	35		X		
Wang et al., (2006)	36	X		X	X
Ma et al., (2006)	37	X		X	
Lukkari et al., (2006)	38	X	X		
Liu et al., (2005)	39	X		X	
Kızılkaya, (2004)	40		X		
Wen et al., (2004)	41	X		X	
Devliegher and Verstraete, (1996)	42	X		X	

To check for publication bias in the datasets, we created funnel plots (Figure S-1). We computed Rosenthal's Fail-Safe N (Rosenthal, 1979) using the *fsn* function of the *metafor* package (Viechtbauer, 2010) in R to determine the number of additional studies for which earthworms do not influence potentially toxic mobility, plant concentration, or uptake that are required to increase the p value to > 0.05. We also computed Fail-Safe N for each group of every moderator for which non-parametric bootstrap 95% confidence intervals lead us to report a significantly different mobility, concentration, or uptake in earthworm-inhabited soils, compared to earthworm free soils (Table S-1).

To quantify the extent to which moderators are associated with one another, we used the *Lambda* function in the *DescTools* package (Signorell et al., 2019) in R to calculate the Goodman Kruskal lambda statistic between each moderator for all four datasets (Tables S-2, S-3, S-4, and S-5). The Goodman Kruskal lambda statistic is a measure of the degree to which the categorisation of observations into groups by one moderator is associated with the categorisation of the observations into groups by another moderator. A value of 0 indicates no association between the two moderators, and a value of 1 indicates a perfect association. Since most moderators have only 4-6 groups (and some have 2 or 3 groups), data with a Goodman Kruskal lambda statistic greater than 0.25 indicates that the meta-analysis of these moderators should be interpreted with caution.

3. Results

As a result of the literature search, we obtained four datasets from a total of 42 studies (Table 1). The first dataset comprised data from 33 studies containing 593 pairwise comparisons (representing 4670 individual measurements) comparing the mobility of potentially toxic elements between bulk earthworm-inhabited soils and soils that were artificially kept earthworm-free. The second dataset comprised data from 11 studies containing 178 pairwise comparisons (representing 1239 individual measurements) comparing the mobility of potentially toxic elements between earthworm casts and either bulk earthworm inhabited soils, or earthworm free soils. The third dataset comprised data from 18 studies containing 286 pairwise comparisons (representing 2028 individual measurements) of the concentrations of potentially toxic elements in the tissues of plants grown in soils that were inhabited by earthworms and soils that were artificially kept earthworm-free. The fourth dataset comprised data from 8 studies containing 128 pairwise comparisons (representing 908 individual measurements) comparing the biomass of plant tissues alongside concentrations of potentially toxic elements, enabling us to calculate the uptake of metals by plants grown in soils that were inhabited by earthworms and soils that were artificially kept earthworm-free. The entire dataset is publicly available (Sizmur and Richardson, 2020).

Our meta-analysis revealed several statistically significant impacts of earthworms on the mobility of potentially toxic elements in soil and their uptake by plants. Most of the individual moderator variables selected made significant ($p < 0.05$) contributions to explaining the variation in the data (Table 2). There were two exceptions to this observation. Whether the plant tissue is aboveground (shoots) or belowground (roots) had no significant influence on the impact that earthworms had on the concentration of potentially toxic elements in plant tissues. Also, soil pH had no significant influence on the impact that earthworms had on the uptake of potentially toxic elements into plant tissues. Overall, when all data is considered (Figure 1) earthworms significantly ($p < 0.05$) increase the mobility of potentially toxic elements in the bulk soil and significantly ($p < 0.05$) increase the concentration of potentially toxic elements in the tissues of plants, with average Cohen's d values of 0.99 and 1.2, respectively. However, the significant ($p < 0.05$) earthworm-induced increase in the mobility of potentially toxic elements in casts (i.e. due to passage through the gut) and uptake into the tissues of plants (i.e. concentration multiplied by biomass) is of a much greater magnitude, with average Cohen's d values of 8.4 and 6.6, respectively.

Table 2 Summary statistics resulting from a random/mixed effects model of the Cohen's d that includes a test for residual heterogeneity (Qe) and a test of moderators (Qm), which represents the statistical significance of the effect that a range of moderators have on the influence of earthworms on (a) metal mobility in bulk soil, (b) metal mobility in casts, (c) metal concentration in plant tissues, and (d) metal uptake into plant tissues.

Dataset	n	Qe	Qm	Moderator	Qm	df	Z	P
(a) Bulk Soil	593	310603	82796	Potentially toxic element	392.3	1	19.8	<0.001
		df = 585	df = 8	Essential/Non-essential element	246.0	1	-15.7	<0.001
		p < 0.001	p < 0.001	Earthworm ecological group	9617	1	98.1	<0.001
				Source of potentially toxic element	89.94	1	-9.48	<0.001
				Type of extraction	60688	1	246	<0.001
				Further amendment	400.4	1	20.0	<0.001
				Soil pH	9115	1	-95.5	<0.001
				Soil organic matter	55.08	1	7.42	<0.001
(b) Casts	178	1546547	351936	Potentially toxic element	30167	1	174	<0.001
		df = 170	df = 8	Essential/Non-essential element	99355	1	-315	<0.001
		p < 0.001	p < 0.001	Earthworm ecological group	5.124	1	2.26	0.0236
				Source of potentially toxic element	9983	1	99.9	<0.001
				Type of extraction	54379	1	233	<0.001
				Further amendment	5011	1	70	<0.001
				Soil pH	27801	1	-167	<0.001
				Soil organic matter	86558	1	-294	<0.001
(c) Plant concentration	286	53277	6034	Potentially toxic element	997.8	1	-31.6	<0.001
		df = 279	df = 7	Essential/Non-essential element	681.6	1	-26.1	<0.001
		p < 0.001	p < 0.001	Earthworm ecological group	20.01	1	4.47	<0.001
				Source of potentially toxic element	35.34	1	5.94	<0.001
				Aboveground/Belowground	0.4721	1	0.687	0.4920
				Soil pH	4531	1	-67.3	<0.001
				Soil organic matter	7.617	1	2.76	0.0058
(d) Plant uptake	128	10327	498	Potentially toxic element	131.0	1	11.4	<0.001
		df = 121	df = 7	Essential/Non-essential element	27.16	1	-5.21	<0.001
		p < 0.001	p < 0.001	Earthworm ecological group	324.8	1	18.0	<0.001
				Source of potentially toxic element	4.010	1	-2.00	0.0452
				Aboveground/Belowground	4.778	1	2.19	0.0288
				Soil pH	0.1592	1	0.399	0.6899
				Soil organic matter	5.747	1	2.40	0.0165

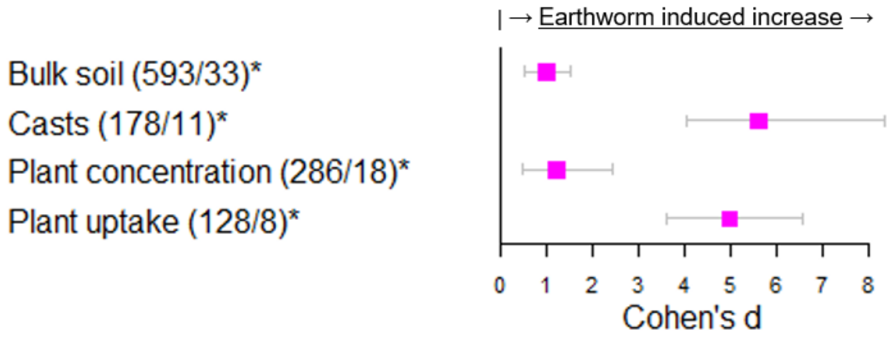


Figure 1 Forest plot depicting mean Cohen's d values representing the influence of earthworms potentially toxic element mobility in soil, mobility in casts, concentration in plant tissues, and uptake into plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

The Goodman Kruskal lambda statistic, while indicating that most moderators were independent (Table S-2, S-3, S-4 and S-5), revealed strong non-independence between soil pH and SOM for all four datasets, since soils with low pH tended to have higher SOM. There was also strong non-independence between SOM and the source of potentially toxic elements in the soil in the plant uptake dataset (Table S-4) because soils that were amended with organic amendments had greater SOM than unamended soils. For all four datasets the Qe statistic was significant ($p < 0.001$), indicating that the effects are heterogenous and that other moderators not considered in the model may influence the effects (Table 2). For the four datasets, Rosenthal's Fail-Safe N was 241711 for potentially toxic element mobility in soil, 11212373 for mobility in casts, 401 for concentration in plant tissues, and 12472 for uptake into plant tissues (Table S-1). Since these Fail-Safe N values are all considerably higher than the number of observations in each dataset, we conclude that there is unlikely to be significant publication bias in our datasets. There were a few moderator groups for which a Fail-Safe

N value of 0 was returned, but this was because our use of non-parametric bootstrapped 95% confidence intervals revealed significant effects whereas Rosenthal's Fail-Safe N reported $p > 0.05$ (so 0 additional observations were required for $p > 0.05$). The overwhelming majority of moderator groups had Fail-Safe N values far in excess of the number of observations. The only group where Fail-Safe N was lower than the number of observations was where pH > 7.5 for the plant concentration dataset.

3.1. Impact of earthworms on potentially toxic element mobility and plant uptake

Overall, our analysis revealed that the mobility of both essential and non-essential potentially toxic elements was significantly greater in bulk earthworm-inhabited soils, compared to earthworm-free soils (Figure 2a), and significantly ($p < 0.05$) greater in earthworm casts, compared to earthworm-free soil (Figure 2b). The earthworm induced increase in potentially toxic element mobility was significantly ($p < 0.05$) greater in casts, compared to bulk earthworm-inhabited soil. The concentration of both essential and non-essential potentially toxic elements in plant tissues and their uptake into plant tissues (i.e. concentration multiplied by biomass) was significantly ($p < 0.05$) greater in plants grown in earthworm-inhabited soils, compared to plants grown on earthworm-free soils (Figure 2c and 2d). Earthworms increased the uptake of non-essential potentially toxic elements (but not essential potentially toxic elements) into plant tissues significantly ($p < 0.05$) more than they increase the concentration in plant tissues.

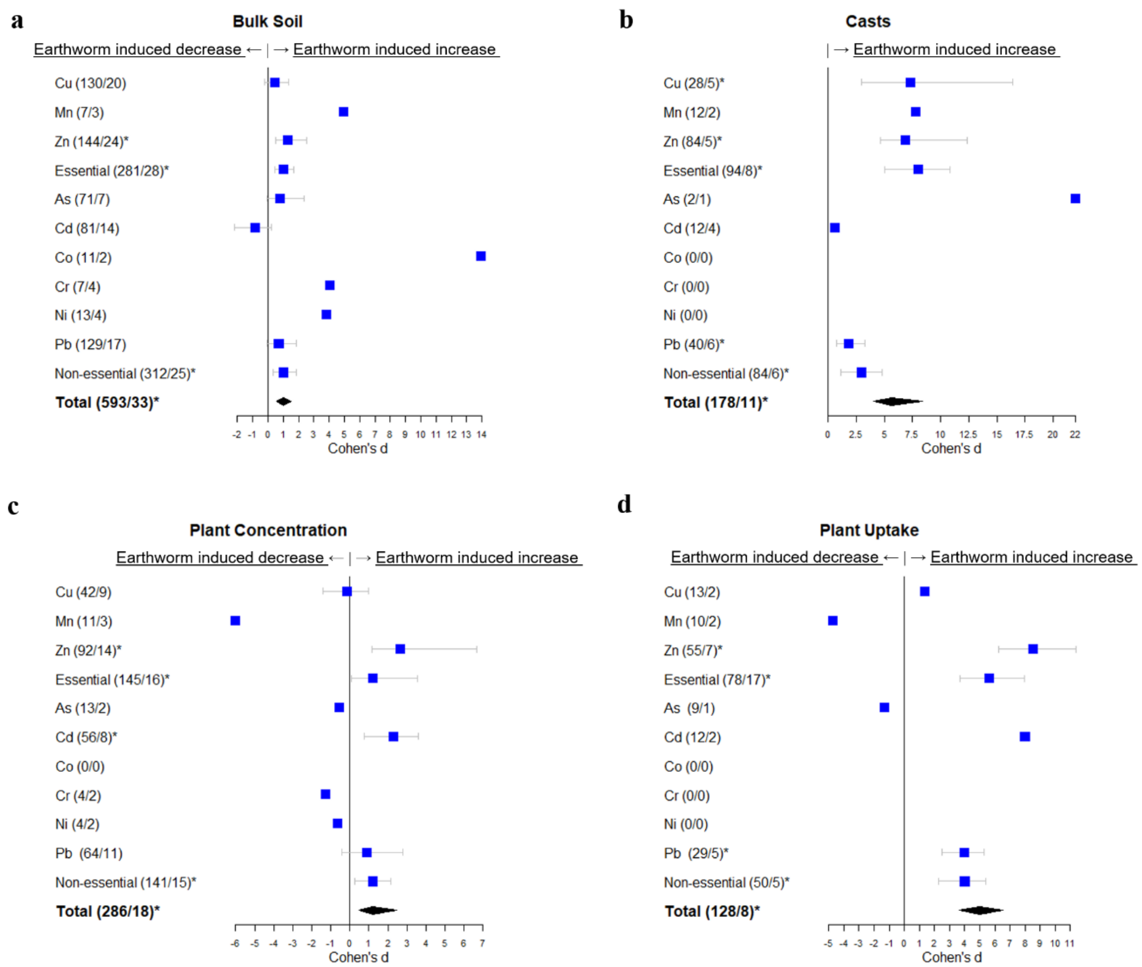


Figure 2 Forest plot depicting mean Cohen's d values representing the influence of earthworms on essential (Cu, Mn and Zn) and non-essential (As, cd, Co, Cr, Ni and Pb) potentially toxic element (a) mobility in soil, (b) mobility in casts, (c) concentration in plant tissues, and (d) uptake into plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

The published data on earthworm induced potentially toxic element mobilisation and uptake by plants focuses primarily on elements commonly associated with mining, smelting, industrial, and urban pollution (Zn, Pb, Cu, Cd and As, in descending order of sample size). Thus, relatively few individual elements were studied with sufficient frequency that they could undergo statistical comparisons to assess the influence of earthworms on their mobility or plant uptake. Nevertheless, we found that earthworms significantly ($p < 0.05$) increased the mobility of Pb, Cu, and Zn in earthworm casts (Figure 2b), but only Zn was significantly ($p < 0.05$) more mobile in bulk soil with earthworms present (Figure 2a). Further, plant uptake of Pb, and Zn was significantly ($p < 0.05$) increased by earthworms (Figure 2d), but only Zn plant tissue concentrations were significantly increased by earthworms (Figure 2c). It is clear from the higher Cohen's d values that earthworms influence the uptake of potentially toxic elements to a greater extent than they influence tissue concentrations. Interestingly, Cd mobility was generally (although not statistically significantly) decreased in bulk earthworm-inhabited soil, compared to earthworm free soil (Figure 2a), but Cd concentration was significantly ($p < 0.05$) greater in in plant tissues grown on earthworm-inhabited soil, compared to earthworm free soil (Figure 2c). The only dataset with a large enough sample size to statistically assess the influence of earthworm on As mobility was bulk soil. Arsenic mobility was greater in soils with earthworms present compared to earthworm free soils, but not statistically significantly.

Earthworms significantly ($p < 0.05$) increased the concentration and uptake of potentially toxic elements in both the aboveground (shoots and leaves) and belowground tissues (roots) of plants (Figure 3). This effect was significantly ($p < 0.05$) greater in belowground tissues than aboveground tissues when considering uptake (Figure 3b). Again, the magnitude of the impact that earthworms have on uptake, particularly in belowground tissues, is far greater than the impact that earthworms have on potentially toxic element plant concentrations.

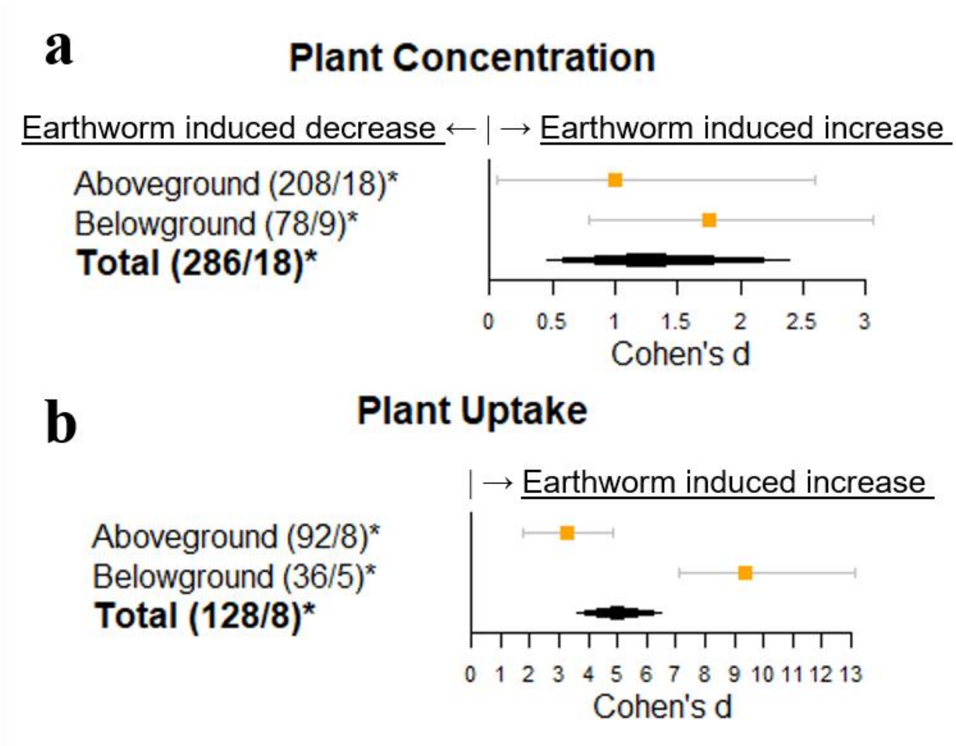


Figure 3 Forest plot depicting mean Cohen's d values representing the influence of earthworms on potentially toxic element concentration (a) and uptake (b) into aboveground and belowground plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

3.2. Influence of different earthworm ecological groups on the mobility and plant uptake of potentially toxic elements

We hypothesized that earthworm ecological group would influence earthworm impact on potentially toxic element mobility and uptake by plants due to their different feeding strategies and where in the soil profile that they deposit their casts. Indeed, earthworms from different ecological groups had different effects on mobility and uptake of potentially toxic elements in the soil-plant system. Endogeic and epigeic earthworms significantly ($p < 0.05$) increased the mobility of potentially toxic elements in bulk soil and casts (Figure 4a and 4b). Epigeic earthworms (but not endogeic earthworms) also significantly ($p < 0.05$) increased concentration and uptake of potentially toxic elements by plants (Figures 4c and 4d). However, while anecic earthworms significantly ($p < 0.05$) increased the mobility of potentially toxic elements in their casts to a greater magnitude than epigeic and endogeic earthworms, with a Cohen's d value of 8.1, their impact on potentially toxic element mobility in bulk soil returned a Cohen's d value of only 0.08, and they were also associated with a non-significant decrease in plant uptake and a significant decrease ($p < 0.05$) in plant tissue concentrations (Figure 4c). Epigeic earthworms significantly ($p < 0.05$) increased potentially toxic element mobility in bulk earthworm-inhabited soil, casts, plant concentrations, and plant uptake (Figure 4), but are overwhelmingly represented by experiments using the compost earthworm *Eisenia fetida*.

3.1. Influence of the nature of contamination on earthworm induced mobility and plant uptake of potentially toxic elements

The majority of experiments undertaken to assess the effect of earthworm activity on the mobility of potentially toxic elements and their uptake by plants have used soils contaminated by mining, smelting, urban, or industrial activities, the application of organic wastes, or soils artificially spiked with salts of potentially toxic elements in the laboratory. Generally speaking, our results indicated that earthworms significantly ($p < 0.05$) increase the mobility and plant uptake of potentially toxic elements under each of these circumstances (Figure 5). Earthworm also significantly ($p < 0.05$) increase the mobility of geogenic potentially toxic elements in bulk earthworm inhabited soil and casts, to a greater extent than in soils anthropogenically contaminated or artificially spiked with potentially toxic elements (Figure 5a and 5b). However, earthworms inhabiting geogenic soils significantly ($p < 0.05$) decrease the concentration and uptake of potentially toxic elements into plant tissues (Figure 5c and 5d).

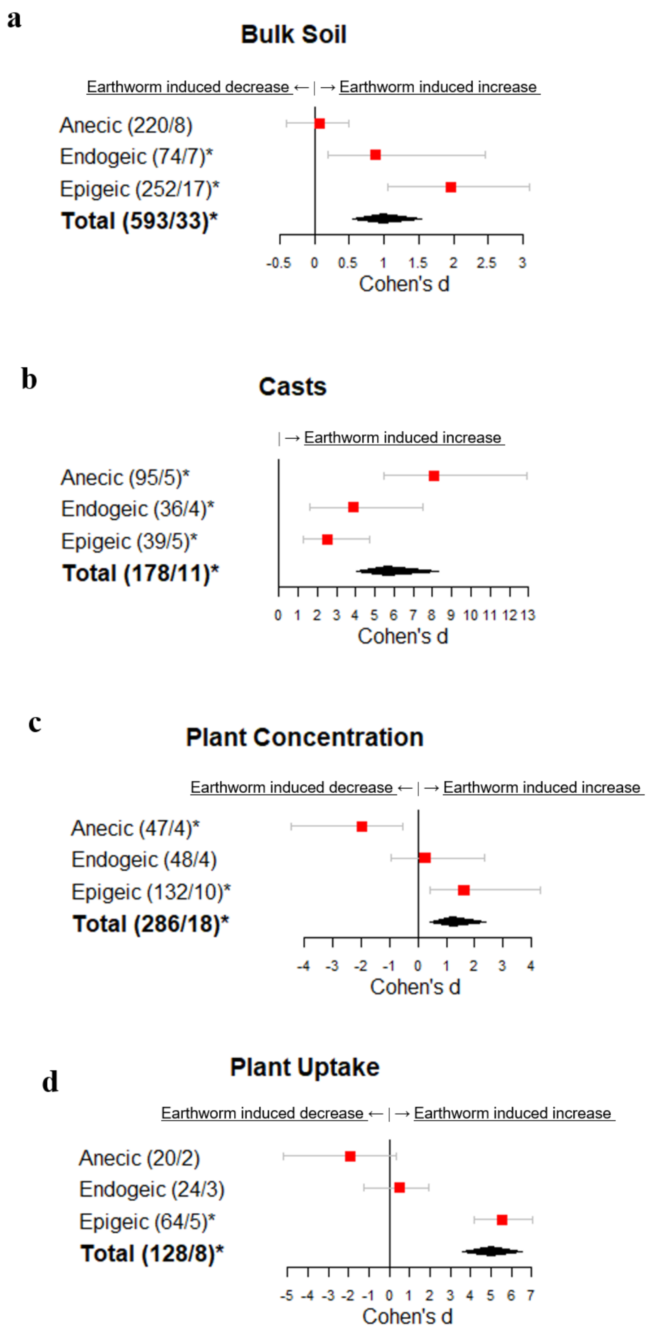


Figure 4 Forest plot depicting mean Cohen's d values representing the influence of earthworms from three ecological groups on (a) metal mobility in soil, (b) metal mobility in casts, (c) metal concentration in plant tissues, and (d) metal uptake into plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

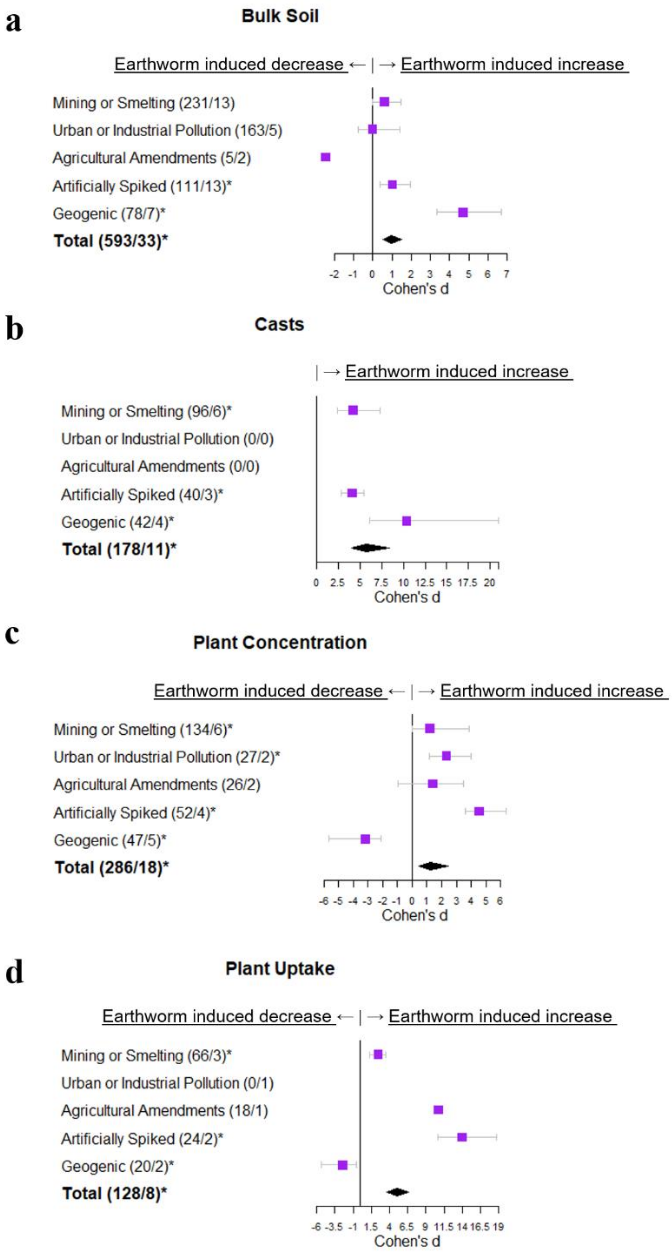


Figure 5 Forest plot depicting mean Cohen's d values representing the effect that the source of potentially toxic elements in soils has on the influence of earthworms on (a) potentially toxic element mobility in soil, (b) potentially toxic element mobility in casts, (c) potentially toxic element concentration in plant tissues, and (d) potentially toxic element uptake into plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

3.2. Influence of experimental design features on earthworm induced mobility of potentially toxic elements

Whereas different publications selected for this meta-analysis adopted different methodologies to quantify mobility of potentially toxic elements, the majority of studies measure porewater, leachate or extract soils with water. Several studies cite as the reason for this decision the statement by Sizmur and Hodson (2009) that “this fraction represents the most available portion of the total metal concentration in soil and it can be stated with a degree of certainty that this fraction is bioavailable”. Earthworms significantly ($p < 0.05$) increase the water soluble potentially toxic elements in both bulk earthworm-inhabited soil and in earthworm casts (Figure 6). The magnitude of the increase in water soluble potentially toxic elements in bulk soil is comparable to that observed when using extractions with chelating compounds (e.g. EDTA or DTPA) or weak acid solutions (e.g. 0.11 M acetic acid) (Figure 6a). However, the magnitude of the earthworm induced increase in water soluble potentially toxic elements in casts is significantly ($p < 0.05$) greater than these more exhaustive extractants (Figure 6b). Salt solution extraction methods typically used to mimic the ionic strength of soil porewater failed to reveal significant changes in potentially toxic element mobility of soils due to earthworms despite all other methods with $n > 20$ revealing significant increases.

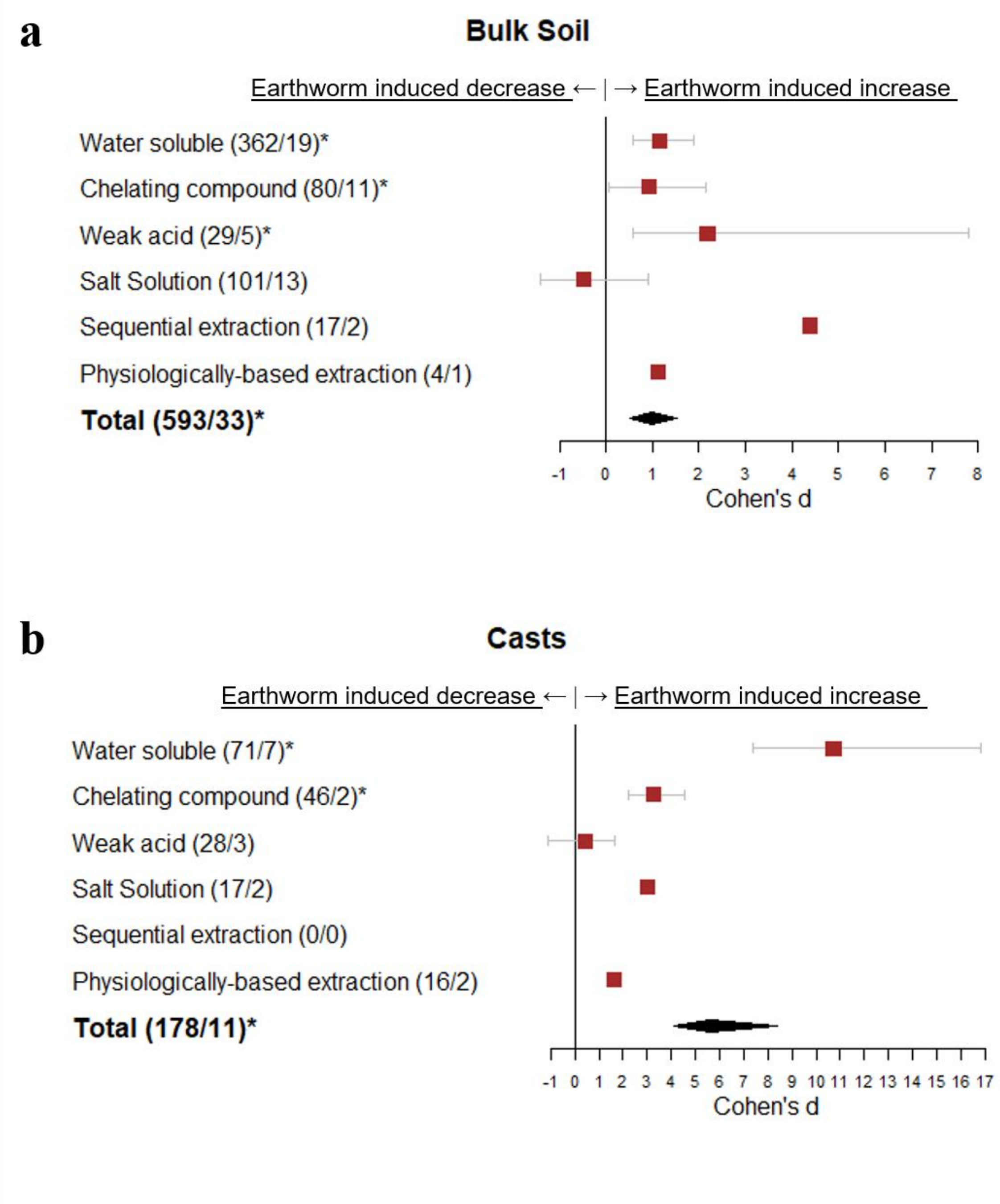


Figure 6 Forest plot depicting mean Cohen's d values representing the effect that the type of chemical extraction that is employed to assess metal mobility in soils has on the influence of earthworms on metal mobility in (a) soil and (b) earthworm casts. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

In several of the experiments selected for this meta-analysis, soils were modified prior to earthworm introduction (the same modification was applied to the earthworm-free control soil). These modifications were generally attempts to 'remediate' soils, either to create a soil that is more hospitable to earthworms, or to investigate the influence of earthworms on the efficacy and longevity of the remediation. Where organic amendments or uncontaminated soil was mixed with contaminated soils, the mobility of potentially toxic elements in the bulk earthworm-inhabited soil is significantly ($p < 0.05$) lower than the earthworm free soil (Figure 7a), although earthworms still significantly ($p < 0.05$) increase mobility in casts of soils mixed with organic amendments (Figure 7b). By contrast, where no amendment was applied the earthworms significantly ($p < 0.05$) increase the mobility of potentially toxic elements in the bulk soil and casts (Figure 7). We were unable to test the influence of amendments on earthworm induced plant uptake of potentially toxic metals since too few studies adopting amendments were obtained.

3.1. Influence of the soil properties on earthworm induced mobility and plant uptake of potentially toxic elements

Experiments included in this meta-analysis utilised soils with a broad range of soil pH and SOM contents (Figure 8 and 9). The meta-analysis did not reveal a consistent pattern regarding the influence of pH of soils on the susceptibility of potentially toxic elements to mobilisation by earthworms (Figure 8a and 8b) or on the plant tissue concentration and uptake (Figure 8c and 8c). Rather, potentially toxic elements in soils at almost all pH levels were susceptible to significant ($p < 0.05$) mobilisation by earthworms (Figure 8). The observations concerning influence of pH on the earthworm induced mobility and plant uptake of potentially toxic elements in this study must be interpreted with caution because there is a strong association between soil pH and SOM, with low pH soils being associated with high SOM (Tables S-2, S-3, S-4 and S-5).

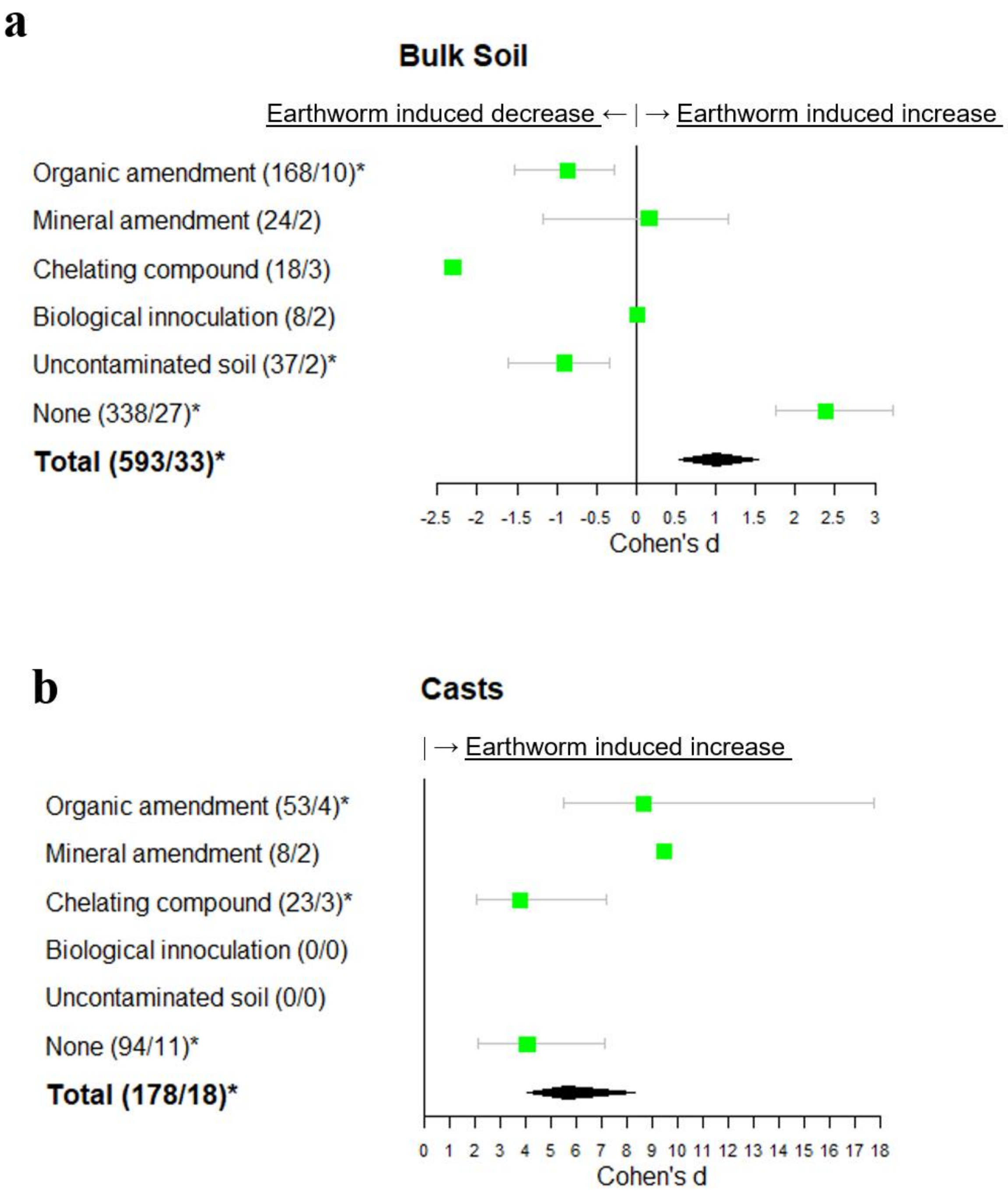


Figure 7 Forest plot depicting mean Cohen's d values representing the effect that further amendments to soils have on the influence of earthworms on metal mobility in (a) soil and (b) earthworm casts. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

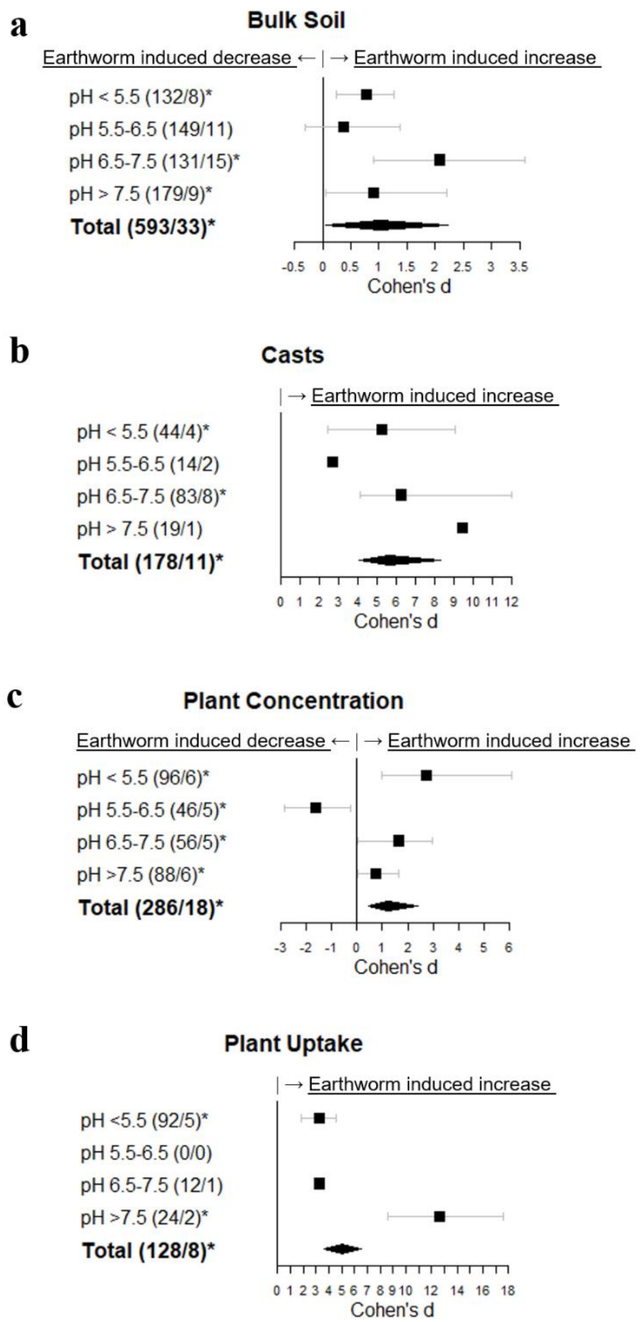


Figure 8 Forest plot depicting mean Cohen's d values representing the effect that Soil Organic Matter (SOM) content has on the influence of earthworms on (a) metal mobility in soil, (b) metal mobility in casts, (c) metal concentration in plant tissues, and (d) metal uptake into plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

We observed contrasting trends in the way that SOM influenced the susceptibility of potentially toxic elements to mobilisation in soils and to uptake by plants (Figure 9). The mobilisation of metals in bulk soil and casts was most pronounced in soils with low (<2%) SOM, with Cohen's d values of 5.0 and 9.4, respectively, and much less pronounced in soils with moderate levels (5-10%) of SOM (Figure 9a and 9b). Nevertheless, potentially toxic element mobility was still significantly ($p < 0.05$) increased at all SOM levels in casts (Figure 9b). The opposite trend is observed in plants. Earthworms significantly ($p < 0.05$) increased plant concentration and plant uptake of potentially toxic elements in soils with moderate levels (5-10%) of SOM, significantly ($p < 0.05$) more than the mobilisation observed in soils with high (>10%) SOM. Earthworms decreased plant concentrations (significantly; $p < 0.05$) and plant uptake (not significantly) of potentially toxic elements in soils with low (<2%) SOM (Figure 9c and 9d), despite significant ($p < 0.05$) mobilisation in casts and bulk soils (Figure 9a and 9b).

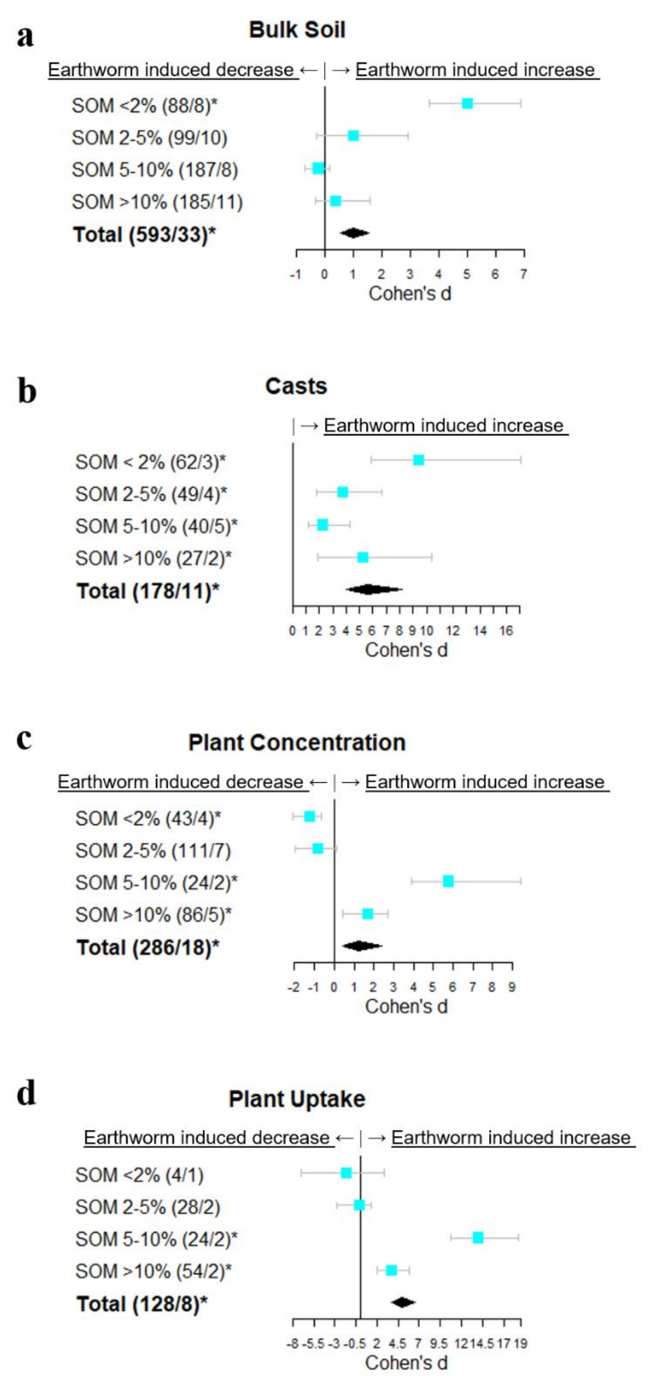


Figure 9 Forest plot depicting mean Cohen's d values representing the effect that soil pH has on the influence of earthworms on (a) metal mobility in soil, (b) metal mobility in casts, (c) metal concentration in plant tissues, and (d) metal uptake into plant tissues. The error bars are bootstrapped 95% non-parametric confidence intervals. The numbers in parenthesis after group labels indicate the number of mean pairwise observations, followed by the number of publications that these observations were drawn from. The * highlights groups where Cohen's d was significantly different from zero at the 95% level.

4. Discussion

4.1. Passage through the earthworm gut increases potentially toxic element mobility

The results of our meta-analysis support our first hypothesis and the conclusion of a previous narrative review (Sizmur and Hodson, 2009) that earthworms increase the mobility of potentially toxic elements in soils and increase their availability for uptake into plants. Endogeic and epigeic (mostly *E. fetida*) earthworms were found to significantly increase the mobility of potentially toxic elements in the bulk soil. This conclusion is also echoed in the findings of several other studies that have been published in the decade since (Sizmur et al., 2011c; Jusselme et al., 2012; Tica et al., 2013; Du et al., 2014; Leveque et al., 2014; Dehghanian et al., 2018; Li et al., 2018), and we therefore conclude that there is a consensus of scientific opinion that, generally speaking, earthworms increase the mobility of both essential and non-essential potentially toxic elements in soils. Furthermore, our results indicate that the magnitude by which earthworms increase the mobility of potentially toxic elements in casts (i.e. due to passage through the earthworm gut) is far greater than in bulk earthworm inhabited soil (Figure 2). This phenomena is particularly clear in datasets presented in Kızılkaya (2004) and (Alonso-Azcárate et al., 2011) for Cu and Zn, and in Sizmur et al. (2011b) for As, Cu and Pb. All three of these studies measure water soluble concentrations in the casts of the anecic earthworm *L. terrestris*. Collecting the casts of anecic earthworms is easier than collecting endogeic or epigeic casts since they typically deposit large casts on the surface of the soil. This difference in feeding and burrowing habits between the three ecological groups (Lavelle, 1997) is most likely the reason why there were more than double the number of observations for anecic earthworm casts (n = 95) than for endogeic (n = 36) or epigeic (n = 39) earthworms (Figure 4).

As soil passes through the gut of the earthworm, it is exposed to a microenvironment with a uniquely cultivated microbiome (Sruthy et al., 2013; Meier et al., 2018) that accelerates the decomposition of organic matter into labile carbon that earthworms can assimilate (Aira et al., 2006; Bernard et al., 2012). The resulting increase in dissolved organic matter can increase the concentration of ions bound to natural organic matter as complexes (Koopal et al., 2005) and increase the mobility of potentially toxic elements in fresh cast material, after passage through the earthworm gut (Wen et al., 2004; Karaca et al., 2010; Sizmur et al., 2011a). Passage through the earthworm gut also mediates soil pH and increases the acid–base buffering capacity of the soil (Kul'bachko et al., 2015), but this typically manifests itself as an earthworm-induced increase in soil pH (Van Groenigen et al., 2019) due to the secretion of calcite granules from the calciferous glands (Lambkin et al., 2011; García-Montero et al., 2013). Therefore, the mediation of pH soil after passage through the earthworm gut is probably not the primary reason why earthworms increase potentially toxic element mobility in soils, but may be a contributing factor to the variability observed in the results of our meta-analysis. Sizmur et al. (2011d) aged *L. terrestris* casts for 56 days and still observed elevated dissolved organic matter and pH in casts, so it is likely that these gut-mediated alterations to soil chemistry persist in the soil for some time and are the primary factor contributing to the increase in potentially toxic element mobility observed in bulk earthworm inhabited soils.

While anecic earthworms clearly increase potentially toxic element mobility in casts, we did not find evidence that they increase potentially toxic element mobility in the bulk soil or increase uptake into plants (Figure 4). In fact, they significantly decrease potentially toxic element concentration in plants. The propensity for anecic earthworms to create permanent burrows as opposed to continually mixing soil horizons may limit their impact on the bulk soil (Jégou et al., 1999; Farenhorst et al., 2000). Because anecic earthworms produce casts in middens on the soil surface (Stroud et al., 2016), these middens, which have greater potentially toxic element mobility, may not be inhabited by roots, which tend to occupy the bulk soil. Furthermore, the permanent vertical burrows of anecic earthworms may enhance the leaching of potentially toxic elements out of soil (Sizmur et al., 2011c), potentially decreasing their availability for plant uptake. Conversely, epigeic earthworms, associated with significantly greater potentially toxic element mobility in bulk soil and casts as well as plant concentration and uptake, may only mix soils within the top few centimetres of soil (Blouin et al., 2013) turning the soil within the whole root zone of an immature plant into cast material and maximising the availability of potentially toxic elements to plants in short-term experimental systems. Epigeic earthworms (particularly *E. fetida*) are typically incubated in the laboratory at a higher temperature than anecic or endogeic earthworms (Lowe and Butt, 2005), and thus ecological group is likely confounded with incubation temperature in this meta-analysis.

4.2. Soils that are most susceptible to earthworm induced increases potentially toxic element mobility and plant uptake

While our overall results support our hypothesis that earthworms increase mobility of potentially toxic elements in soil, increase plant tissue concentrations, and result in greater uptake into plant biomass, when the dataset is divided into groups using moderators, the relationship is far more nuanced. These groups include those based on the soil properties (SOM, pH), whether and how the soil was contaminated, and whether additional amendments were made to the soil. While earthworms increased the mobility of potentially toxic elements in anthropogenically contaminated soils, artificially spiked soils in the laboratory, and geogenic soils, the magnitude of this increase was greatest for geogenic soils (Figure 5). Conversely, earthworms decreased plant concentrations and uptake of potentially toxic elements from geogenic soils (Figure 5). The observations of earthworms reducing plant uptake of potentially toxic elements from geogenic soils are limited to two publications, both of which undertaken using calcareous soils or soils to which calcium carbonate was added to (Bityutskii et al., 2016; Dehghanian et al., 2018) with the objective of using earthworms to alleviate micronutrient stress in calcareous soils. The decrease in uptake is attributed to the breaking of mycorrhizal hyphae that mediate the plant acquisition of micronutrients (Dehghanian et al., 2018), or the greater mixing of calcium carbonate which is able to precipitate metal cations (Bityutskii et al., 2016). Thus, earthworms may exacerbate micronutrient deficiencies in calcareous soil, or after liming. There is also evidence that earthworms decrease the mobility of potentially toxic elements in soils amended with uncontaminated soils or organic amendments (Figure 7). These are often added to highly contaminated soils under experimental conditions to create conditions that earthworms are able to tolerate (Sizmur et al., 2011e). It is likely that in these treatments the earthworms help to mix the contaminated soil with the amendments and increase the adsorption of potentially toxic elements on the surfaces of the amendment and facilitate the remediation process (Ma et al., 2006; Elyamine et al., 2018).

Our results indicate that soils with particularly low SOM (<2%) are most susceptible to earthworm induced increases in potentially toxic element mobility in bulk soil and casts (Figure 9). We hypothesize that the reason why soils with low SOM were more susceptible to earthworm induced metal mobility is because these soils have an inherently lower sorption capacity for metals (Harter and Naidu, 1995; Peijnenburg and Jager, 2003), and so the stimulation of microbial activity as the soil passes through the gut (Cheng and Wong, 2002; Domínguez et al., 2004; Sizmur and Hodson, 2009) results in a proportionately large conversion of SOM to dissolved organic carbon which can bind with potentially toxic elements in solution (Ferraz and Lourençlo, 2000; Sizmur and Hodson, 2009). Relatively modest changes to the chemistry of these soils can result in increases in metal mobility that may be large in proportion but low in concentration (Wen et al., 2004), and thus may not influence plant concentrations or plant uptake to such an extent. Although potentially toxic element mobility was not significantly increased in bulk soils, the greatest increases in plant concentration and plant uptake were observed in soils with SOC concentrations of 5-10%. The two experiments reporting these increases involved the use of *Lantana camara* (Jusselme et al., 2012) and *Brassica juncea* (Wang et al., 2006), two species with known ability to hyperaccumulate metals (Jiang et al., 2000; Liu et al., 2019). Thus, these plants may have been more able to capitalise on the earthworm induced increases in potentially toxic element mobility.

4.3. The Influence of earthworms on the biogeochemical cycling of potentially toxic elements

It is undoubtedly the case that anthropogenic activities have altered the biogeochemical cycling of potentially toxic elements by concentrating them in surface soils during industrial, municipal, and commercial activities (Senesil et al., 1999; Han et al., 2002). Earthworms play a poorly constrained role in accelerating the transport of these elements from contaminated soils into water bodies or into plants and the subsequent food chain. However, each of these elements also has a natural biogeochemical cycle that earthworms contribute to in undisturbed systems. We found earthworms increase the mobility of potentially toxic elements to a greater extent in geogenic soils than in anthropogenically contaminated soils (Figure 5). We also found that earthworms increased plant belowground tissue concentrations of potentially toxic elements more than aboveground tissue concentrations (Figure 3). Belowground plant tissues are more susceptible to potentially toxic element uptake because belowground uptake outside of the Casparian strip is controlled by diffusion gradients, electrochemical gradients, and ion channels while aboveground tissues are limited by more regulated translocation within vascular tissue (Tangahu et al., 2011). Hence, it is likely that the presence of earthworms may increase the plant uptake of micronutrients in deficient soils, but are unlikely to considerably alter aboveground concentrations in soils with optimal micronutrient supply since plants are able to regulate root-to-shoot translocation.

Considering that earthworm populations are generally depressed in cultivated soils (Chan, 2001) and that malnutrition due to micronutrient (e.g. Cu, Zn) deficiencies affects 3.7 billion people, globally (Lal, 2009), land management practices that increase earthworm populations may help increase micronutrient availability to food crops and help to alleviate the 'hidden hunger'. Our findings indicate that the greatest potential for increasing potentially toxic element mobility due to the activity of earthworms is in soils with low SOM (Figure 9). Therefore, earthworms hold the potential to increasing the availability of micronutrients in the most degraded arable soils, where micronutrient deficiencies most often persist.

When earthworms are introduced into ecosystems where they do not naturally occur, they have the potential to considerably alter the chemical (Dobson et al., 2017) , physical (Snyder et al., 2011), and biological (Li et al., 2002) properties of soils. The most widely reported example is the invasion of hardwood forests in the Northeastern United States by exotic species from Europe and Asia (Bohlen et al., 2004; Richardson et al., 2015). However, earthworms have also been deliberately introduced to some ecosystems to improve crop productivity in, for example, pastures in New Zealand and Australia (Stockdill, 1982; Baker et al., 1999). These introductions (deliberate or accidental) are likely to alter the biogeochemical cycling of potentially toxic elements and may increase their availability to organisms at higher tropic levels (Richardson et al., 2015; Richardson et al., 2018). The results of this study indicate that increased plant tissue concentrations and uptake of trace elements, both micronutrients and potentially toxic elements, may occur where earthworms are introduced, depending on the ecological group of the introduced species. However, this is likely to have minimal increases on toxic metal accumulation in foliage and herbivores due to substantial reduction during vascular transport from roots to shoots.

4.4. Observations on the types of studies used in this meta-analysis

There are a number of experimental conditions adopted by the studies cited within this meta-analysis that poorly represent the real interactions between earthworms and potentially toxic elements in the environment. These experimental conditions are often adopted for operational or facultative reasons, usually owing to the difficulty of representing realistic conditions in the laboratory environment and the desire to create statistically meaningful data under externalities that constrain the timeframe and resources within which a project must operate. We therefore highlight a few recommendations for further work to help ensure that future experiments produce data that advances the field.

Several of the experiments considered in this meta-analysis (approximately 1 in 5 observations) were conducted on soils that were spiked with metal salts in order to create an artificially contaminated soil. Spiking soils with metal salts may adequately represent certain types of pollution (e.g. point source or atmospherically deposited) that result in readily mobile potentially toxic elements. However, generally speaking, potentially toxic elements naturally present in soils become mobile as a result of weathering of primary or secondary minerals and potentially toxic elements present as a result of anthropogenic activities typically build up over long periods of time, enabling them to become strongly adsorbed or incorporated within organic matter or secondary oxides (Lu et al., 2005). Therefore, spiking soils with metal salts is usually a poor representation of metal speciation in the real environment (Smolders et al., 2009) and the conclusions that can be drawn from these experiments are limited. Further work should avoid observations on spiked soils.

E. fetida was the most frequently selected earthworm in this meta-analysis. The physiology, life cycles, and ecotoxicological responses of *E. fetida* have been well-characterised, and *E. fetida* is often selected as a model organism for ecotoxicology testing, despite not being a soil-dwelling earthworm

(Spurgeon et al., 2003), primarily due to the ease with which it can be cultured in the laboratory . However, the use of this earthworm to investigate the impact that earthworms have on the mobility and availability of potentially toxic elements in mineral soils skews our overall understanding. *Lumbricus terrestris* and *Lumbricus rubellus* are also well-characterised model species (Bartlett et al., 2010), but there is a lack of information regarding the impact of endogeic earthworms or non-Lumbricidae earthworms (e.g. Megascolecidae) on the biogeochemistry of potentially toxic elements. Future experiments should focus on the impact that these earthworms have on the biogeochemistry of potentially toxic elements.

The vast majority of studies in this meta-analysis focus on the impact that earthworms have in contaminated soils (often highly contaminated soils). Therefore, the findings of individual studies are usually only applicable in the fairly unique setting that the soils are drawn from and there is a limit to the extent to which this information can be applied to the natural biogeochemical cycling of potentially toxic elements in geogenic soils in natural or managed systems. This means that our understanding of the potential for earthworms to alleviate micronutrient deficiencies or alter the potentially toxic element biogeochemistry after the invasion of exotic species to pristine ecosystems is hampered by a lack of observations under these conditions. Furthermore, experiments considered here are generally undertaken in laboratory mesocosms, largely due to the difficulties of establishing earthworm-free control treatments in the field (Wall and Reichman, 2000). However, to advance our understanding, field measurements are essential. While some techniques for manipulating earthworm populations in experimental plots are available (Bohlen et al., 1995; Rhea-Fournier and González, 2017; Keith et al., 2018), the approach of Richardson et al. (2018) in relating potentially toxic element availability and plant uptake to the presence or biomass of particular species in the field is recommended as a way forward.

5. Conclusions

Following a meta-analysis of 1185 pairwise comparisons from 42 peer reviewed journal studies, we find, overall, that earthworms increase the mobility of potentially toxic elements and that this leads to an increase in the uptake and concentration of potentially toxic elements in the belowground and aboveground tissues of plants. We found that endogeic and epigeic earthworms increase the mobility of potentially toxic elements in the bulk soil and earthworms from all ecological groups mobilise potentially toxic elements during passage of soil through the earthworm gut. We also observe an increase in the concentration and uptake of potentially toxic elements by plants growing on soils inhabited by epigeic (mostly *E. fetida*) earthworms. Earthworms increase the mobility of potentially toxic elements in geogenic soils to a greater extent than in contaminated soils and the soils most susceptible to increases are those with very low (<2%) SOM content. Therefore, land management practices that encourage earthworm populations in degraded soils may help to alleviate micronutrient deficiencies. Mixing amendments with contaminated soils with the intention of reducing the mobility of potentially toxic elements may be aided by the activity of earthworms that accelerate the mixing processes. The introduction of exotic species of earthworms into soils may accelerate the natural biogeochemical cycling of potentially toxic elements and increase their availability to higher tropic levels.

Acknowledgements

We would like to thank members of the Soil Biogeochemistry Group at the University of Reading for their helpful and critical comments on this work prior to publication. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. We acknowledge the comments of two anonymous reviewers whose comments have greatly improved the quality of this manuscript.

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Supplementary material for 'Earthworms accelerate the biogeochemical cycling of potentially toxic elements: Results of a meta-analysis'

Tom Sizmur[1]* and Justin Richardson[2]

[1] Department of Geography and Environmental Science, Russell Building, University of Reading, Reading, RG6 6DW, UK

[2] Department of Geosciences, Morrill Science Center, University of Massachusetts, Amherst, MA 01003, USA

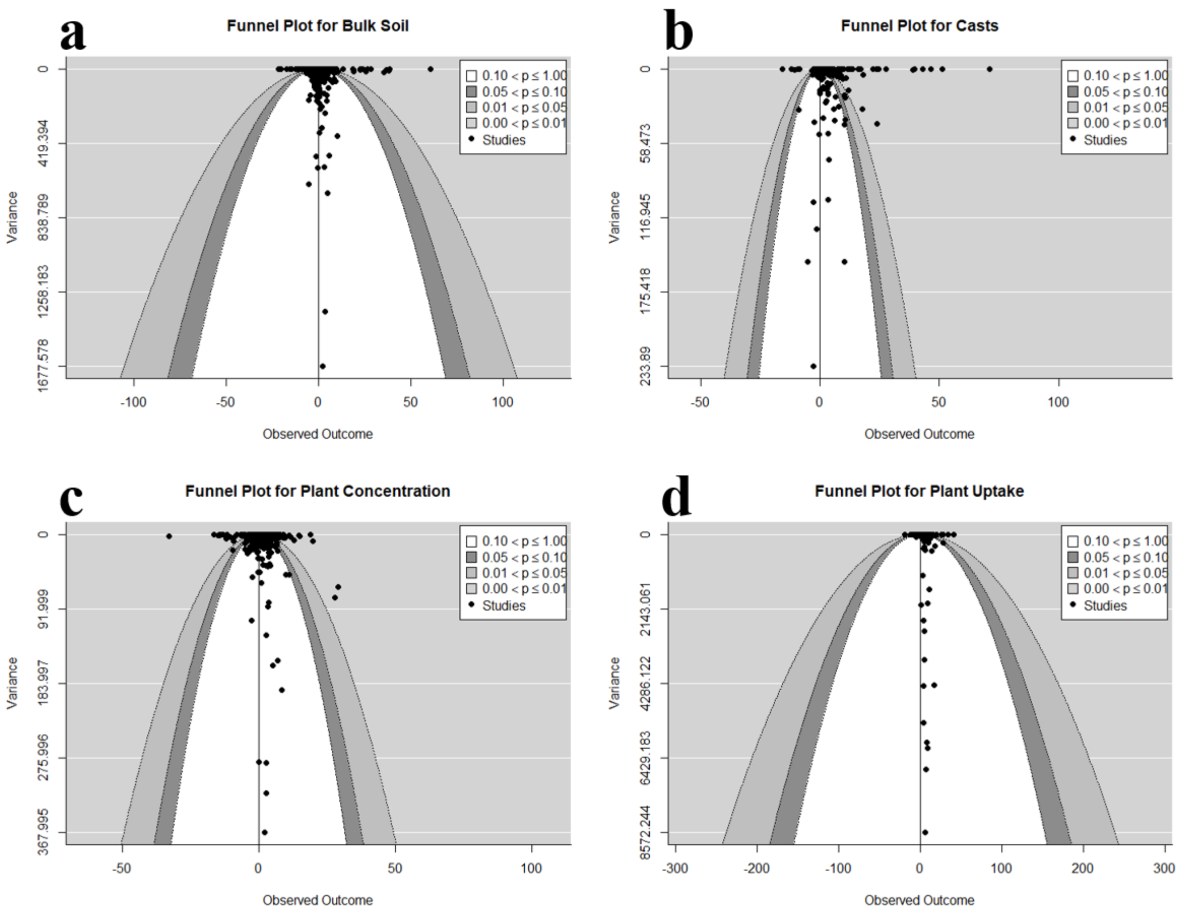

Figure S-1 Funnel plots demonstrating the relationship between the observed outcome (Cohen's d) and the pooled variance for datasets representing potentially toxic element (a) mobility in soil, (b) mobility in casts, (c) concentration in plant tissues, and (d) uptake into plant tissues.

Table S-1 Rosenthal's Fail-Safe N for each of the four datasets (Bulk soil, Casts, Plant concentration, and Plant uptake) and each group of every moderator for which we report a significantly different mobility, concentration, or uptake in earthworm-inhabited soils, compared to earthworm free soils. The Fail-Safe N value is the number of additional observations for which earthworms do not influence potentially toxic mobility, plant concentration, or uptake that are required to increase the significance level to > 0.05.

Dataset	Moderator	Moderator level	Significance level	Fail-Safe N	No. of observations
Bulk Soil			< 0.0001	241711	593
	Potentially toxic element	Zn	< 0.0001	2691	144
	Essential/Non-essential element	Essential	< 0.0001	5161	281
		Non-essential	< 0.0001	320052	312
	Earthworm ecological group	Anecic	< 0.0001	1086	220
		Endogeic	< 0.0001	4505	74
		Epigeic	< 0.0001	247616	252
	Source of potentially toxic element	Artificially Spiked	< 0.0001	18749	111
		Geogenic	< 0.0001	70550	78
	Type of extraction	Water soluble	0.0739	0	362
		Chelating compound	< 0.0001	7341	80
		Weak acid	< 0.0001	163	29
	Further amendment	Organic amendment	< 0.0001	630158	168
		Uncontaminated soil	< 0.0001	1598	37
		None	< 0.0001	314076	338
	Soil pH	pH < 5.5	< 0.0001	4415	132
		pH 6.5-7.5	< 0.0001	130786	131
	Soil organic matter	SOM < 2%	< 0.0001	189311	88
Casts			< 0.0001	11212373	178
	Potentially toxic element	Cu	< 0.0001	2037970	28
		Zn	< 0.0001	2599972	84
		Pb	< 0.0001	20500	40
	Essential/Non-essential element	Essential	< 0.0001	9856902	94
		Non-essential	< 0.0001	43596	84
	Earthworm ecological group	Anecic	< 0.0001	8987017	95
		Endogeic	< 0.0001	21191	36
		Epigeic	< 0.0001	35811	39

	Source of potentially toxic element	Mining or Smelting	< 0.0001	3827424	96
		Artificially Spiked	< 0.0001	3161	40
		Geogenic	< 0.0001	1783607	42
	Type of extraction	Water soluble	< 0.0001	7630674	71
		Chelating compound	< 0.0001	6589	46
	Further amendment	Organic amendment	< 0.0001	1830696	53
		Chelating compound	< 0.0001	34429	26
		None	< 0.0001	3091331	94
	Soil pH	pH < 5.5	< 0.0001	45230	44
		pH 6.5-7.5	< 0.0001	2170341	83
	Soil organic matter	SOM < 2%	< 0.0001	7232787	62
		SOM 2-5%	< 0.0001	38908	49
		SOM 5-10%	< 0.0001	36893	40
		SOM > 10%	< 0.0001	72639	27
Plant Concentration			0.0054	401	286
	Potentially toxic element	Zn	< 0.0001	1172	92
		Cd	0.0014	130	56
	Essential/Non-essential element	Essential	0.2542	0	145
		Non-essential	0.0015	316	141
	Aboveground/Belowground	Aboveground	< 0.0001	8123	208
		Belowground	< 0.0001	4156	78
	Earthworm ecological group	Anecic	< 0.0001	539	47
		Epigeic	< 0.0001	8853	132
	Source of potentially toxic element	Mining or Smelting	0.0621	0	134
		Urban or Industrial Pollution	< 0.0001	1190	27
		Artificially Spiked	< 0.0001	10949	52
		Geogenic	< 0.0001	5323	47
	Soil pH	pH < 5.5	< 0.0001	7764	96
		pH 5.5-6.5	< 0.0001	33919	46
		pH 6.5-7.5	< 0.0001	3388	56
		pH > 7.5	0.0297	**28**	88
	Soil organic matter	SOM < 2%	0.0004	135	43
		SOM 5-10%	< 0.0001	398	24

		SOM > 10%	0.4615	0	86
Plant Uptake			< 0.0001	12472	128
	Potentially toxic element	Zn	< 0.0001	5546	55
		Pb	< 0.0001	248	29
	Essential/Non-essential element	Essential	< 0.0001	3576	78
		Non-essential	< 0.0001	2634	50
	Aboveground/Belowground	Aboveground	< 0.0001	723	92
		Belowground	< 0.0001	6970	36
	Earthworm ecological group	Epigeic	< 0.0001	8625	64
	Source of potentially toxic element	Mining or Smelting	0.0086	73	66
		Artificially Spiked	< 0.0001	4037	24
		Geogenic	< 0.0001	641	20
	Soil pH	pH < 5.5	< 0.0001	1702	92
		pH > 7.5	< 0.0001	3908	24
	Soil organic matter	SOM 5-10%	< 0.0001	4037	24
		SOM > 10%	< 0.0001	4062	54

Table S-2 Goodman Kruskal lambda statistics for the Bulk soil dataset representing the probable improvement of predicting one moderator group from the other moderator group, scaled between 0 and 1.

Bulk soil	Potentially toxic element	Earthworm ecological group	Source of potentially toxic element	Type of extraction	Further amendment	Soil pH
Potentially toxic element						
Earthworm ecological group	0.111					
Source of potentially toxic element	0.068	0.158				
Type of extraction	0.044	0.146	0.022			
Further amendment	0.030	0.233	0.178	0.041		
Soil pH	0.075	0.200	0.380	0.112	0.175	
Soil organic matter	0.139	0.326	0.398	0.075	0.175	0.349

Table S-3 Goodman Kruskal lambda statistics for the Casts dataset representing the probable improvement of predicting one moderator group from the other moderator group, scaled between 0 and 1.

Casts	Potentially toxic element	Earthworm ecological group	Source of potentially toxic element	Type of extraction	Further amendment	Soil pH
Potentially toxic element						
Earthworm ecological group	0.107					
Source of potentially toxic element	0.148	0.133				
Type of extraction	0.124	0.105	0.259			
Further amendment	0.028	0.162	0.247	0.173		
Soil pH	0.081	0.183	0.108	0.148	0.123	
Soil organic matter	0.205	0.317	0.182	0.238	0.175	0.466

Table S-4 Goodman Kruskal lambda statistics for the Plant concentration dataset representing the probable improvement of predicting one moderator group from the other moderator group, scaled between 0 and 1.

Plant concentration	Potentially toxic element	Earthworm ecological group	Source of potentially toxic element	Aboveground/ Belowground	Soil pH
Potentially toxic element					
Earthworm ecological group	0.020				
Source of potentially toxic element	0.107	0.307			
Aboveground/Belowground	0.000	0.000	0.000		
Soil pH	0.169	0.070	0.196	0.030	
Soil organic matter	0.160	0.198	0.226	0.012	0.301

Table S-5 Goodman Kruskal lambda statistics for the Plant uptake dataset representing the probable improvement of predicting one moderator group from the other moderator group, scaled between 0 and 1.

Plant uptake	Potentially toxic element	Earthworm ecological group	Source of potentially toxic element	Aboveground/ Belowground	Soil pH
Potentially toxic element					
Earthworm ecological group	0.015				
Source of potentially toxic element	0.081	0.349			
Aboveground/Belowground	0.000	0.000	0.000		
Soil pH	0.000	0.360	0.367	0.000	
Soil organic matter	0.088	0.290	0.529	0.027	0.472