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## A meta-analysis-based evaluation of metallic element accumulation in earthworms

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### ABSTRACT

The responses of earthworms to excess soil element concentrations are well studied. However, published information on the metallic element accumulation in individuals is controversial. In this paper, the published data on earthworm As, Cd, Cr, Cu, Ni, Pb, and Zn whole body concentrations were evaluated in individuals collected from contaminated and uncontaminated (control) soils, using *meta*-analyses. The role of soil pH and exposure time as potential influencing factors on metal accumulation was also assessed. Based on the evaluations, the accumulation of each metallic element was significantly ( $p < 0.05$ ) more intensive in individuals collected from contaminated soils than in ones from control soils, with minor differences in the order of accumulation intensity among the studied metallic elements. Further, major interspecific differences were indicated in the accumulation, with different species being the most intensive accumulators for individual metallic elements. Among the studied metals, Cu concentration in earthworm bodies increased significantly with increasing soil pH. As for the exposure time-dependent accumulation, Pb concentration was found to decrease significantly with time in whole body tissues of earthworms. These results suggested a high variability in metal- and species-specific accumulation-excretion patterns of earthworms, influenced also by other external factors. Based on the results highlighted in this *meta*-analysis, accumulation schemes raise the need for further analyses involving other additional variables (e.g., soil type, organic matter content, climatic condition) to get a better understanding of element cycle-earthworm relations.

### 1. Introduction

The living conditions of terrestrial organisms can be severely affected by even minor changes in or around their habitats. Being one of the most prevalent stress factors, excess element concentrations in soils have been handled as an issue with many already revealed, but also with still undiscovered mechanisms and patterns (Sharma et al., 2018; Li et al., 2019). Among afore soil components, a group of elements (e.g., metallic elements) poses a potential concentration-dependent risk to the environment, which are frequently referred to as potentially toxic elements (PTEs) in the literature (Antoniadis et al., 2019; Xiao et al., 2022). A rise in the concentration of these elements in soils can trigger various mechanisms in affected species, which can alter the short- and mid-term distribution and long-term evolution of organisms (Oono et al., 2020; Alsherif et al., 2022).

Relevant research perspectives focus on the responses of potentially

applicable species for reflecting changes in metalloid/metal (hereafter referred to as metallic elements) concentration in soil. Such species are called bioindicators (Bayouli et al., 2021). The manifestation of their main characteristics can be various, out of which the increase of concentration in tissues is a common mechanism for a broad range of species (Arora, 2018; Wang et al., 2021). So far, for these purposes, the remediation potential of several hundreds of microorganisms, plants, and animals has been studied (Koul and Taak, 2018). To be good candidates in aiding soil metallic element decontamination, species must possess certain characteristics such as a high degree of tolerance to the contaminating compound and good accumulation potential in their tissues (Yan et al., 2020). Further, fast growth and high abundance are also supporting the success of the remediation process. Most of the studies involve plants for the remediation, but the number of papers evaluating the role of animals in the bioaccumulation of soil elements is also increasing (Józwiak et al., 2019; Nedjimi, 2021).

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An increase in the degree of dependency on the soil itself enhances the applicability of organisms to be reliable agents in soil metal assessments (Parmar et al., 2016). Therefore, earthworms (Annelida: Oligochaeta) are one of the most studied groups of animals regarding metallic element accumulation potential (Gish and Christensen, 1973; Nahmani et al., 2007; Richardson et al., 2020; Yuvaraj et al., 2021b). Many of the earthworm species are abundant and stress-tolerant, which are prerequisites for their good collectability and the opportunity to study a high variety of compounds being bioavailable (Usmani and Kumar, 2015; Yuvaraj et al., 2021a). Metal accumulation in earthworms is thoroughly studied, however, it shows a highly element-, species-, uptake route-, tissue- and external factor-specific pattern (Ireland, 1976; Edwards and Lofty, 1977; Jeyanthi et al., 2016; Mondal et al., 2020; Xiao et al., 2022). Triggered by their direct and close contact with soils, earthworms accumulate these elements primarily by the digestion of soil/media (particles) and secondary via dermal uptake from the pore-water, though the functioning of these pathways can be influenced (Srut et al., 2019; Xiao et al., 2022). It was previously reported that *Aporrectodea caliginosa* and *Lumbricus rubellus* had different uptake patterns for heavy metals, which was explained by their ecological roles (Dai et al., 2004). Leveque et al. (2012) also highlighted this phenomenon between *Eisenia* and *Lumbricus* species, while Kamitani and Kaneko (2007) made similar conclusions among six species from three genera. Besides soil concentrations, other factors such as individual species, uptake-excretion ratios, specific susceptibility to genetic damage, soil pH, and the duration of the contact between the contaminant and the earthworm should also be taken into consideration when the rate of the accumulation is assessed (Richardson et al. 2020; Paul et al., 2022). Further, several earthworm species (e.g., *Eisenia fetida*, *Lampito mauritii*) have been proved to have a high ability to transform metals into mineral-bound compounds by the process of vermicomposting (Goswami et al., 2016). Available metallic element accumulation-related information on selected taxa indicates a certain degree of inconsistency in many cases, which calls for the simultaneous re-analysis of these data. For earthworms, such statistical analyses are scarce; for this purpose, using approaches like *meta*-analysis seems to give a good basis (Tózsér et al., 2017, 2019; Richardson et al., 2020). Results from such studies can draw major comparative findings regarding the general metallic element accumulation in earthworms, and also highlight the species with the most intensive uptake, establishing the ground for future in-depth experiments with the underlined species.

The main objective of this study was to check the inconsistencies presented in previous papers and to unify the judgment of earthworm species by the degree of metallic element accumulation; with these objectives, we assessed the arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) concentrations in earthworm species, based on an extensive literature search, with tools of *meta*-analyses. We aimed (1) to reveal, to what degree these metallic elements accumulated in earthworms when studied together (all the species as one group) and studied by species. Further, we intended to find out (2) if there are any interspecific differences in the influence of soil pH and exposure time on the accumulation of metallic elements. We also investigated, (3) whether the element accumulation is significantly influenced by the change in soil pH, and (4) whether the intensity of accumulation significantly changes with exposure time.

## 2. Materials and methods

### 2.1. Literature scan and data selection criteria

We assembled data for this *meta*-analysis by a literature search in each database of Web of Science performed for the period between 1975 and 2022. During the search we applied the following terms: TOPIC = (earthworm) AND TOPIC = (metal) AND TOPIC = (accumulation). To find all the publications to be involved, we reviewed the reference list of resulted papers as well. Publications were considered appropriate for the

*meta*-analysis in case those reported mean concentrations (in mg kg<sup>-1</sup>, dry matter) with their variances and sample sizes for one or more of the most relevant metallic elements (As, Cd, Cr, Cu, Ni, Pb, Zn) in whole bodies of earthworm species collected from contaminated as well as uncontaminated (control) sample sites.

### 2.2. Statistical analyses

For the *meta*-analysis, we calculated the unbiased standardized mean difference (Hedges' *g*) as a common effect size of the contaminated-uncontaminated comparisons.

$$g = J \frac{\bar{x}_U - \bar{x}_C}{S_{within}} \quad (1)$$

$$S_{within} = \sqrt{\frac{(n_U - 1)S_U^2 + (n_C - 1)S_C^2}{n_U + n_C - 2}} \quad (2)$$

$$J = 1 - \frac{3}{4(n_U + n_C - 2) - 1} \quad (3)$$

$\bar{x}_U$  and  $\bar{x}_C$  mean metal concentration (mg kg<sup>-1</sup>) in earthworms collected from uncontaminated (U) and contaminated (C) soils,  $n_U$  and  $n_C$  sample sizes for earthworms collected from uncontaminated (U) and contaminated (C) soils,  $S_U$  and  $S_C$  standard deviations of the metal concentration in earthworms collected from uncontaminated (U) and contaminated (C) soils.

The *g* values were negative in case metallic element concentration was higher in earthworms from contaminated soils than in individuals from uncontaminated ones. Using subgroup *meta*-analysis, we evaluated the similarities in metal uptake from contaminated soils among different earthworm species; the subgroups were the species with sample size ( $n$ )  $\geq 4$  for individual metallic elements.

To estimate the overall effect and the effects of moderators (earthworm species) we used the random-effects model, which eliminates the limitations of common effect size based on the high variation in experimental conditions, design, and methods employed in individual studies (Borenstein et al., 2009). The main benefit of random-effect models is that their degree of plausibility is higher than that of fixed-effect ones; they attribute the distribution of the effect sizes to real differences among studies instead of assuming sampling error as the single reason for effect size variations (Borenstein et al., 2009). For individual studies we computed more than one single effect size, therefore, we applied a publication-level random effect as a nesting factor in the random-effect models. We considered the mean effect size as statistically significant in case the 95 % bootstrap confidence interval (CI; 999 iterations) did not include zero.

Since the interpretation of results would be different if the effect sizes vary across studies (i.e., if there was heterogeneity), or they are homogenous, we investigated effect size heterogeneity across studies. To describe the heterogeneity of effects between studies, we performed complementary heterogeneity measurements ( $Q$ ,  $T^2$ , and  $I^2$ ) (Borenstein et al., 2009). Using a  $Q$ -test, we divided total variance ( $Q_{total}$ ) into within-group ( $Q_{within}$ ) and between-group ( $Q_{between}$ ) variances based on the analysis of variance; these components of variance were tested for statistical significance (Borenstein et al., 2009). Significant between-group variances ( $Q_{between}$ ) mean that the metal accumulation in earthworms was significantly different among species. Additionally, by the calculation of  $R^2$ , we assessed the proportion of true variance explained by covariates (Borenstein et al., 2009). As a general criterion, subgroups with less than four comparisons were excluded from the analyses. Publication bias resulting from missing studies and effect size biases are

also quite general in *meta*-analyses. To assess publication bias we used funnel plots and performed Egger's test (Borenstein et al., 2009). Further, in case of significant asymmetry, we used the trim and fill method, which calculates the number of missing studies, simultaneously computing their effect sizes and standard errors (Duval and Tweedie, 2000). The dataset of the *meta*-analysis was then complemented by found missing studies, which was followed by the re-computation of the summary effect size. With this method, an unbiased estimate of the summary effect size could be given (Borenstein et al., 2009).

Meta-analyses, heterogeneity measures, and publication bias assessments were performed using the *MAd* and *metafor* packages (Viechtbauer, 2010; Del Re and Hoyt, 2014) in the R programming environment (version 4.1.2; R Core Team, 2018). To assess soil pH- and exposure time-related accumulation in earthworms we studied the relationship between Hedges' *g* (standardized mean difference) and soil pH, and between Hedges' *g* and exposure time, respectively. Relationships were assessed with linear models employing the *lm* method, performed in the R.

### 3. Results

#### 3.1. Literature scan

The literature search resulted in 616 publications, complemented by 186 publications from other sources; out of these, 42 papers fulfilled the involvement criteria previously set (Fig. 1 and Supplementary Materials A). From the selected publications, we extracted a total number of 848

comparisons. Reported concentrations of metals in contaminated soils varied greatly across studies (1.02–195 mg kg<sup>-1</sup> for As, 0.1–350 mg kg<sup>-1</sup> for Cd, 5.11–527 mg kg<sup>-1</sup> for Cr, 6.5–15,300 mg kg<sup>-1</sup> for Cu, 4.42–13,926 mg kg<sup>-1</sup> for Ni, 5.5–72,074 mg kg<sup>-1</sup> for Pb, and 25.2–96,800 mg kg<sup>-1</sup> for Zn. In the papers involved, 12 earthworm species were studied (Supplementary Materials A).

#### 3.2. Accumulation of metallic elements in earthworms – Assessment with all species combined

The accumulation of each metallic element (As, Cd, Cr, Cu, Ni, Pb, and Zn) was significantly higher in earthworms from contaminated soils than in ones from control soils. Accumulation intensities for studied metallic elements were similarly high, however, the following accumulation intensity order could be drawn: Pb > Cr > As > Ni > Cd > Cu > Zn (Fig. 2, Supplementary Materials B).

#### 3.3. Accumulation of metallic elements in earthworms – Assessment by species

The accumulation of As was studied in *Dendrobaena octaedra* and *L. rubellus*, and was found (insignificantly) higher in individuals from contaminated soils than those from uncontaminated ones for both species (Fig. 3, Supplementary Materials C1 and D1). Seven species were studied in terms of their Cd accumulation; among these *A. caliginosa* was labeled as the best accumulator, followed by *E. fetida* and *L. rubellus* with also significant uptake intensity compared to control individuals (Fig. 3,

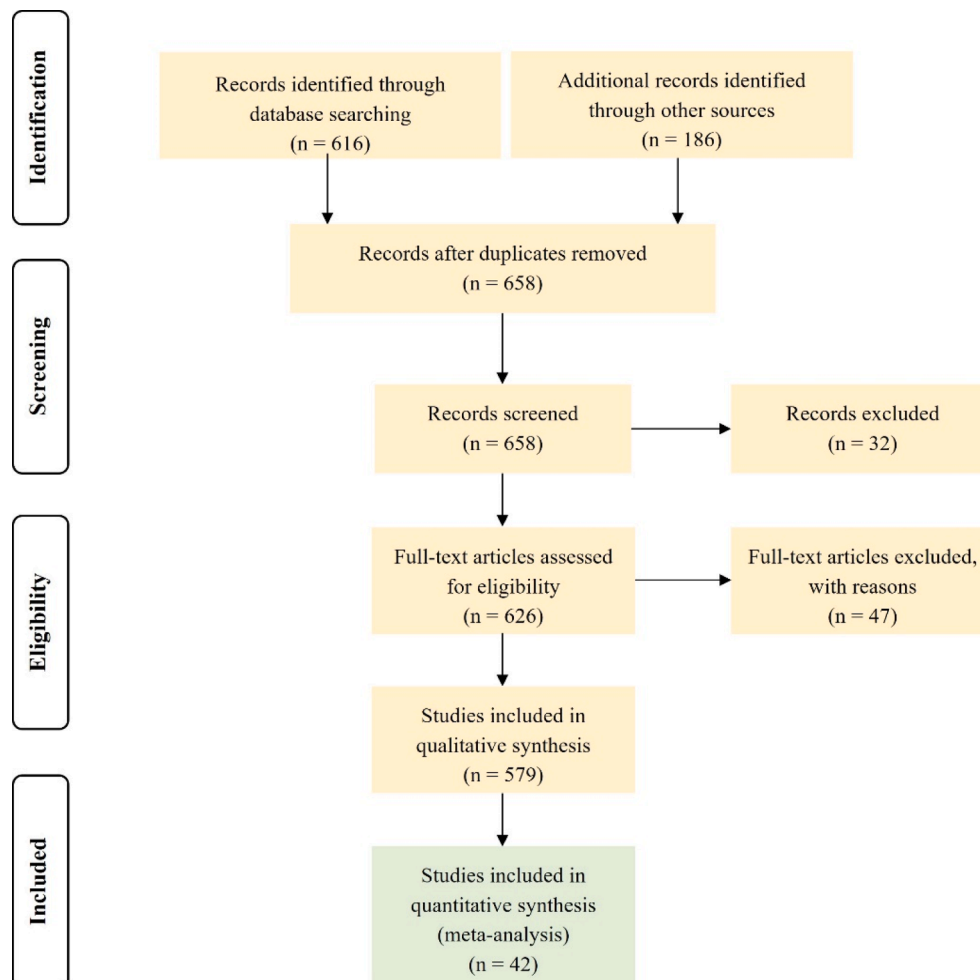


Fig. 1. PRISMA flow diagram of the publication selection process through the analysis (i.e., the number of studies identified, screened, excluded, and included).

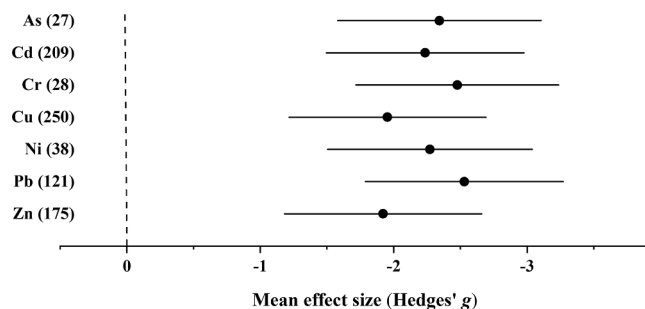


Fig. 2. Mean effect sizes of random-effect models (mean Hedges'  $g \pm 95\%$  CI) for the accumulations of As, Cd, Cr, Cu, Ni, Pb, and Zn in earthworms (all species treated together). Negative  $g$  values represent higher element concentration in earthworm bodies collected from contaminated soils than in individuals from uncontaminated ones. The mean effect size was considered statistically significant if the  $95\%$  bootstrap confidence interval (CI) did not include zero (mean Hedges'  $g \pm 95\%$  CI are entirely in the negative or the positive range). (In brackets: number of comparisons for which the mean effect sizes were calculated.).

Supplementary Materials C2 and D2). Two species were assessed based on their Cr accumulation; *L. rubellus* showed higher accumulation intensity than *E. fetida* in contaminated soils than in uncontaminated ones (Fig. 3, Supplementary Materials C3 and D3). The accumulation of Cu was studied in eight species, out of which *Dendrodrilus rubidus*, *Eisenia andrei*, *E. fetida*, and *L. rubellus* had significant accumulation intensities; however, that for *A. caliginosa*, *Aporrectodea tuberculata*, *D. octaedra*, and *Dendrobaena veneta* was insignificant, but also high (Fig. 3, Supplementary Materials C4 and D4). Among the three studied species for Ni accumulation, *D. octaedra* and *E. fetida* had higher accumulation intensities than *A. caliginosa*, however, all these differences were insignificant (Fig. 3, Supplementary Materials C5 and D5). In the case of Pb, two (*A. caliginosa* and *E. andrei*) of the seven studied species showed significant accumulation. Additionally, *E. fetida* was the species with the lowest accumulation potential (Fig. 3, Supplementary Materials C6 and D6). As for Zn, three (*A. caliginosa*, *D. rubidus*, and *L. rubellus*) of the nine species had significantly higher concentrations in contaminated soils than in uncontaminated ones. Interestingly, the accumulation in *L. terrestris* was significantly lower in individuals from contaminated soils than in those from control ones (Fig. 3, Supplementary Materials C7 and D7).

### 3.4. Effects of soil pH on metallic element accumulation in earthworms

Effects of soil pH could be studied for As, Cd, Cr, Cu, Ni, Pb, and Zn accumulation of earthworm individuals of all species combined. Assessing the accumulations of the individual metallic elements, a significant effect of soil pH was found only in one case (insignificant increase in the accumulation with increasing soil pH: As, Cd, Ni; insignificant decrease in the accumulation with increasing soil pH: Cr, Pb, Zn; Supplementary Materials E). The results of the analyses indicated that the Cu accumulation intensity increased significantly with the increase of soil pH values (Fig. 4, Supplementary Materials E).

### 3.5. Effects of exposure time on metallic element accumulation in earthworms

Effects of the exposure time (test duration) were studied for Cd, Cr, Cu, Ni, Pb, and Zn concentration in earthworm individuals of all studied species treated together. The analyses resulted in only one significant relationship between the accumulation rate and the exposure time (insignificant increase in the accumulation with increasing exposure time: Ni; insignificant decrease in the accumulation with increasing exposure time: Cd, Cr, Cu, Zn). The Pb accumulation rate in earthworms decreased significantly with time, showing significantly lower body

concentrations with the duration of exposure (Table 1, Supplementary Materials F).

## 4. Discussion

In this paper we found differences in the accumulation among metals in earthworms, suggesting a high metal- and species-dependent variability in uptake patterns. The reason for these phenomena can be manifold and requires highlighting, especially in cases of statistically significant ( $p < 0.05$ ) relations.

### 4.1. Accumulation of As by earthworms

The accumulation of As was significant in earthworms. However, studying the two species with adequate sample sizes, we observed higher, but insignificant accumulation potential in the afore relation for both *D. octaedra* and *L. rubellus*, without interspecific differences. Further, soil pH and exposure time did not influence As accumulation significantly.

Fischer and Koszorus (1992) reported that the elimination of As was limited from earthworm bodies complemented by an efficient sequestration mechanism, which can also be a strategy against predators. Additionally, from soil to earthworms, As accumulation is known to be highly dependent on the forms and complexes it occurs in (Lee and Kim, 2008). The forms of As in papers used in this meta-analysis were not identified, while in more cases, tests were run under multi-element-contaminated conditions; the combined effect of these elements did not reduce its accumulation potential in earthworms. It was previously shown that a decrease in soil pH can increase the mobile fraction of metallic elements in soil, making them more bioavailable for uptake in earthworms (Spurgeon et al., 2006). It is in accordance with our observations, although we found a weak correlation between As-uptake and soil pH. Just like for other soil elements, the bioavailability of As for earthworms was reported to be hindered by several other factors, among others the time of exposure (Lee and Kim, 2013), interaction/substitution mechanisms with other soil compounds (Langdon et al., 2003), and the presence of other soil (moisture) compartments such as microplastics (Wang et al., 2019).

Studies comparing As uptake in and among earthworm species are scarce, while (besides papers involved in this study) data are available mainly on other species than the two afore. Assessing As-uptake in *E. andrei*, Kilpi-Koski et al. (2019) concluded an As-specific kinetics, in which both accumulation and elimination take place very slowly, with an effective sequestration mechanism instead. Wang and Cui (2016) found major As-accumulation in *E. fetida*, underlining that applied As forms in the soil also show different accumulation rates and pose demethylation-based toxic risks for earthworm species. In their study Langdon et al. (1999) found much lower As concentration for *L. rubellus* than that in the soil it inhabited; the authors mentioned a high degree of resistance/adaptation as one of the main reasons behind the low body concentration.

### 4.2. Accumulation of Cd by earthworms

Earthworms from contaminated soils accumulated significantly more Cd than individuals from uncontaminated soils. Further, there were also significant differences in the uptake among the seven studied species. Soil pH and exposure time did not have a significant effect on Cd-accumulation.

Richardson et al. (2020) emphasized that Cd is one of the most intensively accumulated metals by earthworms. Further, the detoxification of Cd in earthworms takes place in a very efficient manner, having deposited and neutralized in chloragogenous tissues, and thus reaching high body concentrations without facing lethal consequences (Hussain et al., 2020). Interestingly, earthworms were found to increase soil pH significantly with their presence, which is a factor to consider in

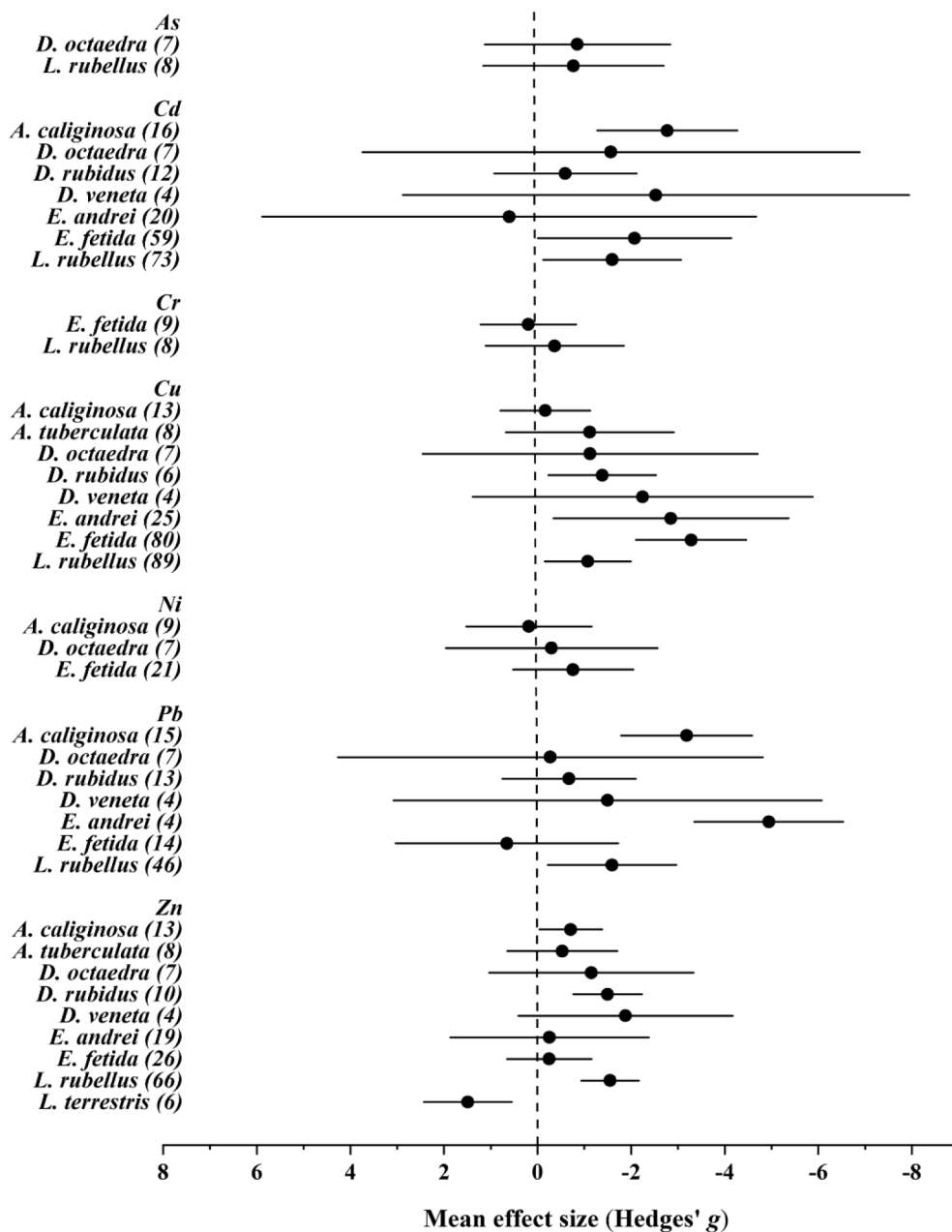


Fig. 3. Mean effect sizes of random-effect models (mean Hedges'  $g \pm 95\%$  CI) for the accumulations of As, Cd, Cr, Cu, Ni, Pb, and Zn in the studied earthworm species. Negative  $g$  values represent higher element concentration in earthworm bodies collected from contaminated soils than from uncontaminated ones. The mean effect size was considered statistically significant if the 95% bootstrap confidence interval (CI) did not include zero (mean Hedges'  $g \pm 95\%$  CI are entirely in the negative or the positive range). (In brackets: number of comparisons for which the mean effect sizes were calculated.).

future studies (Wen et al., 2004). As a further influence, contrasting our findings, most of the relevant studies reported a slow, but constant increase in earthworm body Cd concentration with the time of exposure, with no equilibrium reached in moderately and highly contaminated media due to restricted excretion (Elyamine et al., 2018; Urionabarretxea et al., 2020); this, at the same time, gives a basis why we found high Cd-accumulation when all species were combined.

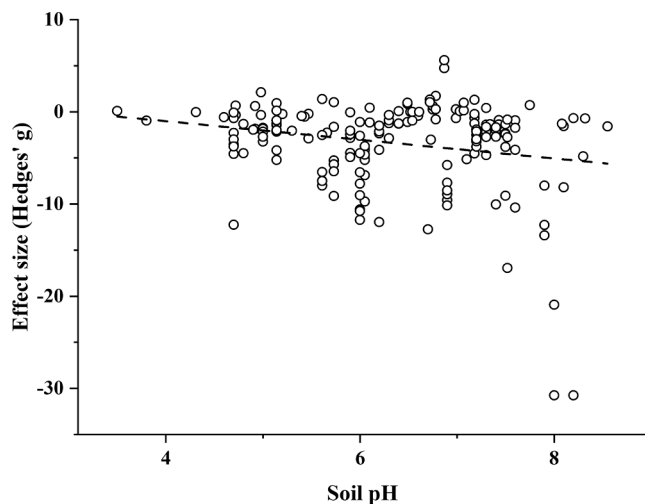
In general, uptake of Cd in earthworms shows a species-specific pattern. In accordance with our results, Latif et al. (2013) found very intensive Cd-uptake in *A. caliginosa*, the species with the best Cd-accumulation potential in this study. Similarly, significant Cd-accumulation was identified for *E. fetida* (Łapiński and Rosciszewska, 2008), which was also supported by our analysis. On the other hand, it was also demonstrated that in some species (e.g., *E. fetida*) the expression of metallothionein, the protein accountant for the Cd-detoxification down-regulates, causing varying accumulation and sequestration patterns (Goswami et al., 2016). Contrasting our results Giska et al. (2014) emphasized non-intensive accumulation and low excretion rates for

*L. rubellus*. Removal of body Cd was found to be more efficient by other species such as *E. andrei* (González-Alcaraz et al., 2018), which contradicts our observation finding the species the least favorable accumulator of Cd. Our results on the role of soil pH in Cd-uptake are also different from previously reported ones: we found no major correlations, while diverse effects of decreasing soil pH on the uptake in *L. rubellus* (Oste et al., 2001), and bioavailability-based significant effects for *A. caliginosa* (Perämäki et al., 1992) were previously reported.

#### 4.3. Accumulation of Cr by earthworms

The accumulation of Cr was significantly higher in earthworms from contaminated soils than ones from control conditions. There was no significant difference in the low accumulation rates of *E. fetida* and *L. rubellus*, while the effects of pH and exposure time were of minor importance regarding Cr-accumulation in earthworms.

As a Cr-specific feature, the speciation of Cr should also be taken into consideration by its toxicity, thereby for the accumulation and



**Fig. 4.** Relationship between the standardized mean difference (Hedges'  $g$ ) calculated for Cu concentration in earthworms collected from contaminated vs uncontaminated soils and the soil pH. More negative  $g$  values indicate higher difference in Cu concentration (greater accumulation intensity) in earthworm individuals from contaminated vs uncontaminated soil ( $F = 8.8$ ,  $n = 177$ ,  $p = 0.003$ ,  $R = -0.22$ ).

**Table 1**

Relationship between the standardized mean difference (Hedges'  $g$ ) calculated for Pb concentrations of earthworms collected from contaminated vs uncontaminated soils and the exposure time ( $n = 40$ ).

Component of variance	$d.f.$	Sum of squares	Mean square	F value	$p$ value
Model	1	98.776	98.776	4.566	0.039
Error	38	822.087	21.634		
Total	39	920.863			

detoxification (Sivakumar and Subbhuraam, 2005). Further, the organic matter content of the soil that organisms inhabit plays a vital role in the bioavailability of Cr, which has major importance in the case of earthworms (Balasoiu et al., 2001). As for the analysis with the species treated together, we detected no significant effect hindering Cr uptake in the studied group of annelids.

Compared to our results on all earthworm species, previous findings are rather contrasting. However, species-specific accumulation patterns can be discovered. As a factor supporting the success of accumulation, Hartenstein et al. (1981) observed that the growth and survival of *E. fetida* were not affected even by an extremely high contamination level ( $46,000 \text{ mg kg}^{-1}$ ) of the soil. Due to slow accumulation and efficient excretion rates, *E. fetida* was characterized by low internal Cr concentration in the study of Di Carlo et al. (2020); the authors also denoted that body Cr concentration changes were independent of soil pH values. Low Cr uptake rates were demonstrated also by other authors, mentioning soil multi-contamination and metal interactions as potential limiting factors by the accumulation of the metal (Aleagha and Ebadi, 2011; Bozym, 2017). Accordingly, Kilpi-Koski et al. (2019) presented fast, but low uptake intensity for *E. andrei*, with no major increase in body concentration already from the second day of exposure. Only a minor body concentration increase was reported for *E. fetida* from the 21<sup>st</sup> to the 42<sup>nd</sup> day of exposure, which suggested an equilibrium in Cr concentration reached in earthworms within a brief period (Mostafaei et al., 2016). It was also reported that suffering life cycle impairments, *E. fetida* was able to withstand high Cr exposure with increasing Cr concentration in its body (Nirola et al., 2018). Similar to our results van Vliet et al. (2006) found insignificant accumulation in *L. rubellus* in contaminated soils, although the species had higher body concentrations

than other ones (e.g., *A. caliginosa*) involved. We assume that the uptake potential of species with small sample sizes (*A. caliginosa*, *Alma millsoni*, *Aporrectodea rosea*, *D. rubidus*, *L. mauritii*) in the analysis of all species combined masked the low degree of applicability of individually studied species.

#### 4.4. Accumulation of Cu by earthworms

Out of the studied metallic elements, uptake of Cu was significantly intensive in earthworms. We found significant differences among the eight studied species as well. Soil pH significantly influenced Cu accumulation in earthworms, while exposure time had no major effect on the uptake pattern.

In the literature high (Delgado Arroyo et al., 2014) and low (Bamidele et al., 2015) Cu accumulation rates for earthworm species have also been reported. It was noted as an early observation that earthworms usually avoid soils contaminated by Cu, which narrows the opportunities for assessing their accumulation patterns (Rhee, 1969; Hartenstein et al., 1980b). Unlike in our study, most authors presented (in many cases significant) increase in body Cu concentration with decreasing soil pH, explained by the enhanced bioavailability of the metal under acidic conditions (Vijver et al., 2007). Assessing different soil types (e.g., organic material supply) and Cu-loads, the accumulation-favoring effect of soil pH decrease can be suppressed (Tatsi et al., 2018).

In their paper Hartenstein et al. (1980a) emphasized that an elevated soil Cu concentration promotes a more intensive accumulation in *E. fetida* than under less Cu-contaminated conditions. This latter checks up with our results on the accumulation of studied species. In accordance with our findings, Van Gestel et al. (2009) referred to *L. rubellus* as a great Cu-accumulator in an extensive study on the species. Further, van Vliet et al. (2006) found *L. rubellus* to be a much better accumulator of Cu than *A. caliginosa*, which was based primarily on differences in food preference and physiology. Species-specific regulation (excretion) of body Cu was also indicated to lower tissue concentration, resulting in low body concentrations detected (Veltman et al., 2007). The above characteristics of earthworms can be responsible for the observation, that some species are capable of accumulating Cu in high concentrations despite its low mobility and bioavailability in soils (Dai et al., 2004). We presume that these interspecific diet and lifestyle characteristics explain our results on the influence of soil pH on Cu accumulation rate.

#### 4.5. Accumulation of Ni by earthworms

The accumulation of Ni was significantly higher in earthworms from contaminated soils than in individuals from uncontaminated ones. Furthermore, there were also significant interspecific differences among the three studied species. Soil pH and exposure time did not influence earthworm tissue Ni concentration.

Besides papers involved in this study, information on Ni accumulation in earthworms are scarce in comparison to other metallic elements. Similar to our findings, Podolak et al. (2011) observed high accumulation potential in earthworms from contaminated sites, explained primarily by their inefficient Ni-regulation mechanism. Further, high Ni concentrations were reported to have an only minor effect on adult earthworm mortality rates, thus the opportunity for accumulation remained high (Lock and Janssen, 2002).

Slow excretion of Ni in *E. fetida* was reported by Yan et al. (2011), who also emphasized the differences in accumulation among organs. On the other hand, Hirano and Tamae (2010) reported no uptake of Ni by *E. fetida*, labeling the metal as non-bioaccumulable. In addition, toxicity symptoms and thereby accumulation patterns shows high interspecific variability (e.g., *Eisenia* species), which makes the evaluation of Ni accumulation a complex issue (Scott-Fordsmand et al., 1998). Tischer (2012) also noticed that, in line with Cu accumulation, the uptake of Ni represents a highly specific pattern dependent on the ecophysiological preferences of earthworm species, soil characteristics, and individual

test setup conditions. We found that *E. fetida* was the most susceptible Ni accumulator species out of the three studied ones, however, without significant accumulation potential. As a further indication of the low accumulation, Peijnenburg et al. (1999) found a fast, but restricted Ni uptake in *E. andrei*, without a major increase with exposure time, which suggests an internal threshold-based excretion pattern in the case of the species.

#### 4.6. Accumulation of Pb by earthworms

Both the enrichment of earthworm tissues in Pb and related interspecific differences in accumulation among the seven included species were significant. Further, body concentrations were independent of soil pH, while exposure time significantly influenced the intensity of uptake in individuals with internal concentration drop as time passed.

Unlike the results in our study, Dai et al. (2004) found the uptake of Pb the least intensive in earthworms compared to the accumulation of Cd, Cu, and Zn. For this reason, efforts have been made to find agents that foster the permanent accumulation of Pb in earthworms (Zhang et al., 2014). Previously it was also proved that interactions among metals highly influenced the bioavailability/accumulation pathways of Pb, which, in the presence of plants, can result in the restricted accumulation of the metal in earthworms (Weltje, 1998). In contrast, we found a general significant accumulation by the species when analyzed as one group, behind which the high variability of experimental conditions in papers included is supposed.

Also, in contrast with our findings, the authors indicated very low uptake rates for both *A. caliginosa* and *L. rubellus*, interpreted as a result of the low degree of solubility and bioavailability of soil Pb-compounds. Further, Van Hook (1974) presented only up to 20 % of the soil concentration in bodies of surrounding earthworm individuals. Leveque et al. (2012) came to the same conclusion, underlining the low bioavailability of Pb, while finding *A. caliginosa* a much better accumulator than *L. rubellus*, primarily due to their differences in vertical position (lifestyle) in soils. Tang et al. (2017a) explained the low Pb accumulation in *E. fetida* by its metal-specific metabolism pattern and the faster excretion than in cases of other metals. Further, Hartenstein et al. (1980a) underlined the importance of exposure time in the case of Pb accumulation in *E. fetida*; the authors noted that the tissue concentration increment rates for Pb required were much lower than those for other metals. In another study Tang et al. (2017b) found that by certain Pb forms and soil types, metabolic responses of *E. fetida* could not enable an efficient excretion, allowing a major growth in body concentration with time. This latter, at the same time, contradicts our results showing a significant decrease in body Pb concentration as time passes. In addition, Darling and Thomas (2005) observed a pronounced decrease in the bioavailability of certain Pb-compounds such as  $Pb(CO_3)_2$  with time, which, along with the excretion of Pb by *L. terrestris*, resulted in a decrease in body concentration.

#### 4.7. Accumulation of Zn by earthworms

Significant accumulation was shown for Zn in earthworm body tissues, while differences among the nine studied species were also significant. There were no major influences of soil pH and exposure time on the accumulation patterns investigated.

Concentration-dependent Zn regulation of earthworms and the form of the metal are primary and species-dependent driving forces influencing body concentrations in earthworms (Laycock et al., 2016). As an essential element, Zn shows a specific accumulation pattern in earthworms: the intensive enrichment is followed by maintaining a concentration equilibrium state, which both are also affected by metal speciation and interspecific accumulation differences (Ardestani et al., 2014; Swati and Hait, 2017).

Similar to our findings, Xiao et al. (2020) also reported strong interspecific differences in Zn accumulation for earthworms. In their

paper, Lev et al. (2010) showed concentration-dependent uptake of Zn in *L. terrestris* and found stunted development and condition deterioration by high Zn levels, which support our findings on this species with the lowest accumulation potential among all. In moderate doses and bioavailable forms, Zn was found to accumulate in high concentrations in *L. terrestris* and their casts, with no reported condition drop of the individuals (Kızılkaya, 2004). In line with our observations, besides their Cd accumulation potential, Dai et al. (2004) named *A. caliginosa* and *L. rubellus* as the species with very high Zn uptake. Similarly, Hashemi et al., (2018) indicated particularly good Zn accumulation in *E. fetida* body, which contradicts our results; however, the authors pointed at the formation of strongly modified elemental accumulation patterns in earthworms living in multi-contaminated soils. In this paper, we suppose this latter as a potential explanation for the low uptake rates in *E. fetida*, as many of the involved papers studied soils with multi-contamination schemes.

## 5. Conclusions

This meta-analysis-based assessment of the metallic element accumulation potential of earthworm species indicated highly element- and species-specific patterns. All the studied metallic elements (As, Cd, Cr, Cu, Ni, Pb, and Zn) accumulated significantly ( $p < 0.05$ ) in earthworm bodies. Most of the individual species had significantly high accumulation potential, but huge interspecific accumulation differences also appeared. Additionally, soil pH and exposure time were major influencing factors; significantly intensive accumulation was indicated for Cu in soils with high pH, while significant internal tissue concentration drop was also presented for Pb with time. The outcomes of this analysis demonstrated major differences in metallic element accumulation, which was based on the various metal- and species-specific accumulation and excretion characteristics. The effects of these parameters, complemented by the involvement of other external factors (e.g., soil type, organic matter content, climatic condition), should also be taken into consideration in future accumulation analyses, which would support a closer assessment of element cycles in earthworms.

Uncited references

Hashemi et al., 2018; Laycock et al., 2016.

## CRedit authorship contribution statement

**Dávid Tózsér:** Conceptualization. **Szabolcs Mizser:** Conceptualization. **Katalin Karaffa:** Visualization. **Hajnalka Málík-Roffa:** Visualization. **Tibor Magura:** Methodology.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2022.107546>.

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