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The Effect of Soil Amendments on Trace Elements' Bioavailability and Toxicity to Earthworms in Contaminated Soils

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Abstract: The aim of this study was to assess the impact of soil amendments, characterized by different sorption properties, on the effectiveness of trace elements' (Cu, Zn, Pb, Cd, Ni, and Cr) stabilization and bioavailability to earthworms. The study was conducted as a microcosm experiment using soil derived from a heavily contaminated post-industrial area. The *Eisenia veneta* earthworm was cultured for 4 weeks in soils amended with materials characterized by different properties, origins, and potential effects on limiting the availability of metals in soils: two type of compost (Zabrze compost-ZC; GWDA compost-GC), two types of biosolid (Bełchatów biosolids-BB, Grabów biosolids-GB), calcium phosphate (CP), iron oxide (IO), bentonite (BE), rock waste (RW), and limestone (CC). After the incubation, the biomass and survival numbers of the earthworm species decreased significantly ($p < 0.05$). The accumulation of metals in the earthworm tissues expressed by the bioaccumulation factor value (BSAF) were dependent on the type of amendment applied to the soil. The highest decrease in the earthworms' weight and survival rate was caused by compost (72%) and bentonite (33%), while the lowest was caused by the rock waste (10%) and iron oxide (11%). The biosolids exhibited the greatest toxicity, causing the mortality of all the earthworms. The accumulation of metals in earthworm tissues and the BSAF value were dependent on the type of amendment applied to the soil. The BSAF for the contaminated soil by Cd decreased to the greatest extent after the addition of ZC (by 57%), GC (55%), CP (41%), and IO (37%). A similarly positive effect was noted for Pb after IO addition (45% decrease). The Zn, Cr, and Ni concentration in earthworms, contrary to other elements, increased, regardless of the amendment. The results showed that the applied soil amendments were characterized by varying potential for the reduction in the metal bioavailability in the soil, depending on their composition and physicochemical properties. Moreover, earthworms may exhibit a diversified response to soil amendments as a result of the impact of amendment on the metal forms in soils and their direct impact on organisms. Generally, the Cd was easily transferred from the soil into and accumulated in the earthworm tissues. Our study confirms that this element creates the highest risk for the trophic chain in soils affected by the Zn and Pb smelting industry. Moreover, greater Zn supply reduces the accumulation of Cd in animal bodies. This study provides valuable practical knowledge on the short-term biological effects of a range of soil amendments in metal-contaminated soils.

Keywords: metal immobilization; cadmium; zinc; lead; copper; nickel; chrome; bioassay; BSAF; compost; biosolids

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1. Introduction

Soil contamination with trace elements (TE) is a common problem in terrestrial ecosystems as a result of their dispersion. It is related to urbanization and industrial activities, such as mine tailings, disposal of high metal wastes, leaded gasoline and paints, the application of fertilizers to land, animal manure, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, the spillage of petrochemicals, and others [1–3]. TE cover a group of inorganic elements, including potentially hazardous metals commonly found at contaminated sites in concentrations exceeding their permissible contents [4–6]. Although the presence of TE in soil originates from parent-rock material, TE might be converted to mobile forms due to changes in environmental conditions or soil properties. Their mobile forms may be available to living organisms [7].

The bioavailability of TE in soils is considered a dynamic process comprising three distinct steps: (1) environmental availability, i.e., the total amount of TE in the soil, including both actual and potential fractions, which can be dissolved from the soil matrix into the pore water; (2) environmental bioavailability, i.e., the amount of dissolved fraction in the pore water that can be taken up by plant roots or other soil organisms; and (3) toxicological bioavailability, i.e., the amount of TE that can physiologically induce bioaccumulation or other effects within plants depending on translocation, metabolism, and degradation [3,8–10]. All these approaches to bioavailability are strongly affected by soil physicochemical properties [3,7,10–13]. Soil components imply the sorption processes and control TE behavior because soils differ greatly in their sorption capacity, their cation and anion exchange ability, and the binding energies of their sorption sites. The sorption of metals in soil may be very fast (from 5 to 15 s), but the kinetic rates depend on the properties of the TE [11]. The strength of metal binding and retention may be additionally caused by many other soil factors, such as pH, the nature of the soil's components, the presence and concentration of organic and inorganic ligands, root exudates, and nutrient concentrations [2,3,10,11,14,15].

Among different remediation approaches, in situ and ex situ processes, including excavation, solidification, stabilization, soil washing, electroremediation, and phytoremediation, have been used to mitigate the negative impact of metals in soils [2,15–18]. To achieve effective reductions in the TE availability in soil, various amendments are proposed in the scientific literature [2,14,15,17,19–21]. Chemical stabilization is a valuable strategy for a wide range of contaminated sites. TE stabilization can be carried out using materials of organic and inorganic origin characterized by diversified sorption affinity and influence on soil properties [2]. The application of inorganic soil stabilizers, including lime, phosphate materials, zeolite, bentonite, sepiolite, or Fe/Mn oxides, is based on their ability to shift soil pH, precipitate insoluble TE minerals, occlude TE, or enhance their binding, thus leading to a longer-lasting remediation effect [2,10,17,22,23]. Organic materials, such as biosolids, composts, manure [14–16,18,19,21], and biochar [20], have attracted significant attention from researchers as potential high-performance materials due to their considerable sorption affinity with a wide range of soil contaminants. The search for the most effective soil amendment is ongoing due to the high potential for the immobilization of TE in the soil and the associated low risk of releasing metals during their aging processes.

The recovery of contaminated soils in highly polluted areas is necessary to prevent the deterioration of the environment, as well as to control the exposure to hazardous and toxic compounds for humans and other living organisms. The development of effective tools to accurately predict the bioavailable fraction of metals constitutes a very important step in providing a risk assessment of the potential exposure of soil-dwelling organisms and can contribute to improvements to ecosystem health, as well as in remediated ecosystems. The methods for the measurement of TE availability are based on different techniques, including chemical extraction [9,10,12,13,21,24,25], modeling processes [26,27], and bioassays [7,8,12,13,21,24,28,29]. Of these, bioassays give a more direct measurement of biological responses, as well as the real bioaccumulation rate of TE [8,12,13,21,29–31]. The direct responses of organisms exposed to potentially polluting elements obtained by bioassays are indicative of the actual risk of pollutants [32]. Although these methods are time-consuming and relatively expensive (due to the different endpoints, exposure times, and uptake mechanisms involved), they are commonly used as efficient predictors of metal bioavailability [7,12,13,21,24,25]. For this purpose, earthworms are considered to be the most important bio-indicators due to their intimate (external and internal) contact with the soil and the possibility they offer of evaluating the potential of TE bioaccumulation and food-chain transfer [7,13,31,33,34]. Studies published in recent years pointed out that the variation in the TE bioaccumulation in worms may be dependent on their bioavailability [35–40], it is as affected by soil factors influencing metal solubility (pH and redox) and complexation with organic matter [41–43] However, these factors and mechanisms are still poorly understood. Moreover, earthworm studies in contaminated areas have mainly focused on the analysis of the assimilation of TE by earthworms in artificially contaminated soils. Relatively few studies have been conducted to examine the effectiveness of soil amendments at reducing TE bioaccumulation in soil organisms.

Therefore, the aim of our research was to assess the impact of soil amendments characterized by different sorption properties and mechanisms (two types of compost, two types of biosolid, calcium phosphate, iron oxide, bentonite, rock waste, and limestone) on the level of TE (Cu, Zn, Pb, Cd, Ni, and Cr) bioavailability for earthworms. The research was conducted as a microcosm experiment using soil derived from heavily contaminated area subjected to long-term industrial pressure.

2. Materials and Methods

2.1. Experimental Design

The research was conducted using highly contaminated [6] soil (soil P) subjected to long-term anthropopressure related to the intensive activity of Zn and Pb smelting industry. Soil was sampled from Upper Silesia region in southwestern part of Poland (Piekary Śląskie: 50.3789° N, 18.9270° E), from an area exposed to strong impact of dust deposition (Cu, Zn, Pb, Cd, Ni, Cr). Collected soil was identified as loamy sand, characterized by 60 g kg^{-1} of organic matter content and alkaline pH at level of 7.6. The concentration of individual metals in soil was diversified with predominant content of total Zn and Pb and significantly lower concentrations of other metals, such as Cd, Cu, Ni, Cr (Zn = 4090 mg kg^{-1}, Pb = 2200 mg kg^{-1}, Cd = 146 mg kg^{-1}, Cu = 28.8 mg kg^{-1}, Ni = 12.0 mg kg^{-1}, Cr = 10.8 mg kg^{-1}, respectively). However, soil contamination with Cd was still extremely high when compared to background values in uncontaminated soils. Additionally, the uncontaminated [6] soil (soil UP) of similar texture was collected from the IUNG-PIB Experimental Station, located in eastern area of Poland (Osiny: 51.4642° N, 22.0712° E), as "blank" soil in the experiment with earthworms to determine reference-metal bioaccumulation. Soil UP was specified as loamy sand with 19.7 g kg^{-1} of organic matter and pH value at 6.9.

The research was conducted based on the microcosm laboratory experiment in which two main parameters, solubility and bioavailability of TE, were analyzed. For this purpose, the 1.5 kg of soil P was amended by one of exogenous organic or mineral materials (soil amendments) described in Section 2.2. The soils thoroughly mixed with the amendments were incubated for 30 days at 20–22 °C, at moisture equivalent to 70% of the field water holding capacity (FWHC). After incubation, the samples were divided into 3 sub-samples (replicates) by placing 0.5 kg portions of soil in 1-liter glass jars. Earthworms were then introduced into each jar (5 individuals per jar) and stored in the dark at room temperature (20 °C) for 28 days (4 weeks). Soil moisture was adjusted to 70% of FWHC every three days.

The aim of the experiment was to determine the total and bioavailable metal fraction that was absorbed directly by earthworms. Therefore, after the end of the bioassay period, the survival and change in weight of earthworms, as well as soil physicochemical properties, were evaluated (Sections 2.2.1 and 2.2.2).

2.1.1. Soil Amendments

Soil amendments applied in the experiment included nine exogenous organic or mineral materials (Zabrze compost-ZC, GWDA compost-GC, Bełchatów biosolids-BB, Grabów biosolids-GB, calcium phosphate-CP, iron oxide-IO, bentonite-BE, rock waste-RW, limestone-CC), characterized by different properties, origins, and potential for limiting the availability of TE in soils.

Detailed description of the applied soil amendments, as well as of the dose of their application, is provided below, while the physicochemical properties of the multicomponent amendments are summarized in Table 1:

1. Zabrze compost (ZC): Produced in Zabrze in pile-composting process, based on a range of biodegradable-waste types (green waste, kitchen waste, and municipal biosolids) (dry compost dose equal to 10% of soil mass);
2. GWDA compost (GC): Produced by GWDA Piła in a pile-composting process using mixture of biosolids, food waste, municipal green waste, and agricultural biodegradable waste as a substrate (dry compost dose equal to 10% of soil weight);
3. Bełchatów biosolids (BB): Produced in a municipal sewage treatment plant in Bełchatów (dry sludge dose equal to 10% of the soil mass);
4. Grabów biosolids (GB): Produced in the small municipal sewage treatment in Grabów on Pilica river (dry sludge dose equal to 10% of soil mass);
5. Calcium phosphate (CP): Added to soil as reagent-grade calcium phosphate $CaHPO_4$ (dose of phosphorus equal to 2% of soil weight);
6. Iron oxide (IO): Added to soil as amorphous iron oxide in the form of reagent grade $Fe(OH)_3$ (a dose of iron equal to 2% of soil weight);
7. Bentonite (BE): A fossil aluminium phyllosilicate clay, consisting mostly of montmorillonite (dose of dry matter equal to 5% of soil weight);
8. Rock waste (RW): Rock waste from mining process with a high content of clay minerals (dry matter dose of material equal to 10% of soil mass);
9. Limestone (CC): Added to soil as reagent-grade calcium carbonate (a dose of calcium carbonate equal to 10% of the weight of the soil).

Rates of amendments were selected based on those applied for remediation studies of heavily metal-contaminated soils in the past [2,10,19]. We also adopted the principle that amendment doses cannot create physically difficult conditions in the soil. Rates of all organic amendments were unified to 10% of the soil weight. CP and IO were added as doses equivalent to approx. 4 t ha^{-1} of P and Fe, respectively. Bentonite and RW rates (5 and 10% of soil weight, respectively) were set arbitrarily, taking into account the clay content in these materials, which was much greater in bentonite.

Table 1. Physicochemical properties of the soil amendments.

Soil Amendments	Abbrev.	pH	Total Concentration						
			OM	P	Fe	Mn	Cd	Zn	Pb
			[%]	[%]		[mg kg^{-1}]			
Zabrze compost	ZC	7.4	36.3	0.60	1.79	1290	4.1	816	350
GWDA compost	GC	5.3	43.9	0.20	2.58	500	0.9	1600	77
Bełchatów biosolids	BB	6.7	54.3	0.21	3.54	490	5.5	1890	91
Grabów biosolids	GB	6.1	75.1	0.39	0.84	450	1.7	1650	29
Bentonite	BE	9.0	-	0.30	0.81	189	0.05	85	35
Rock waste	RW	6.5	-	0.13	0.92	110	<0.05	89	36

OM—organic-matter content; pH—soil acidity; P—phosphorus; Fe—iron; Mn—manganese; Cd—cadmium; Zn—zinc; Pb—lead.

2.1.2. Earthworms

Earthworms are common and important soil invertebrates, and in comparison with other soil organisms, they are sensitive to soil pollutants due to their close interactions with soil and thin cuticle [8]. Therefore, the *Eisenia veneta* earthworm was selected as the tested organism due to its rapid growth, life cycle, and high compostability parameters, according to the literature data [44].

2.2. Sample Analysis

2.2.1. Soil Analysis

The soils were subjected to analysis of physical and chemical parameters prior to the experiment. These included soil texture, pH, and organic-matter concentration. The pH was measured potentiometrically in a 1:2.5 (m V^{-1}) soil suspension in H_2O (PN-ISO10390, 1997). The particle size distribution was analyzed via the aerometric method (PN-R-04032, 1998), while soil organic matter content was determined after sulfochromic oxidation followed by titration of the excess of $K_2Cr_2O_7$ with $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (PN-ISO 14235, 2003).

Trace-element concentrations (Zn, Pb, Cd, Cu, Ni, Cr) were determined by the aqua regia digestion (0.5 g air-dried, sieved through 2-millimeter mesh and ground on mortar-ground soil), with involvement of middle-pressure (32 bars) microwave digestion system (Mars Xpress from CEM Corp., Matthews, NC, United States). The extracts were analyzed by the inductively coupled plasma mass-spectrometry technique (Agilent quadrupole 7500CE ICP-MS equipped with a torch, micro mist nebulizer, nickel sampler and skimmer cones, and double-pass spray chamber). Argon was used as a carrier gas, and hydrogen and helium as reaction gases for the elimination of interferences. To minimize the matrix effect and ensure long-term stability, all determinations were made in the presence of internal standard consisting of 1 mg L^{-1} of 45Sc, 89Y and 159Tb. A blank sample and the certified reference material (CRM028-050) were included in analyses for quality control. The recovery for analyzed trace elements was from 90 to 97%, while precision of the method defined as a relative standard deviation (RSD) was <3%. The LOD values were determined at the level 0.085 mg kg^{-1}, 0.081 mg kg^{-1}, 0.007 mg kg^{-1}, 0.050 mg kg^{-1}, 0.032 mg kg^{-1}, 0.099 mg kg^{-1}, respectively, for Zn, Pb, Cd, Cu, Ni, Cr.

The soluble forms of metals (Zn, Pb, Cd, Cu, Ni, Cr) were analyzed using 0.01M calcium nitrate ($Ca(NO_3)_2$) extraction (liquid-to-soil ratio = 1:2.5) in an end-over-end shaker for 2 h. The metal concentrations in the extracts were determined by the inductively coupled plasma mass-spectrometry technique, as described above.

2.2.2. Earthworm Analysis

After the end of the bioassay, earthworms were counted and weighed. Subsequently, they were transferred into Petri dishes with moist filter paper. Next, they were kept at 25 °C for 48 h and filter papers were changed daily to allow full depuration of earthworms. Earthworms were then killed in liquid nitrogen and further oven-dried at 100 °C for 24 h. Trace-element content in earthworms was determined after digestion of sample with concentrated HNO_3 at 140 °C for 3 h, followed by 1 mL of H_2O_2 with re-heating for 2 h at 140 °C. TE concentration in extracts from earthworms were determined by ICP-MS using the same method as soil samples.

The response of earthworms to soil contamination was evaluated in terms of relative toxicity and through bioaccumulation indices reported by "survival rate" (SR), "weight gain" (WG), and "biota-soil accumulation factor" (BSAF) [28]. The weight change was expressed in normalization to the number of surviving earthworms. The factors were calculated based on the following equations:

$$SR = \frac{S}{S_{tot}} \tag{1}$$

$$WG = \frac{E(5)W_{Initial}}{E(5)W_{Final}} \times 100\% \tag{2}$$

$$BSAF = \frac{TE_{ET}}{TE_{SOIL}} \tag{3}$$

where S—the number of earthworm survivors; S_{tot}—total initial number of earthworms; $W_{Initial}$—sum of weights of five earthworms used in the experiment in individual jars; W_{Final}—sum of weights of five earthworms after the incubation in individual jars; TE_{ET}—

trace-element concentration in earthworm tissue; TE_{SOIL}—total trace-element concentration in soil P (soil contaminated by TE).

2.3. Statistical Analysis

The software package Statistica (Dell Statistica, Stat Soft version 13.3) was used for statistical analysis. Basic statistical parameters, such as mean, standard deviation, and coefficient of variation (CoV) were calculated. One-way analysis of variance (ANOVA) with the Tukey post hoc test (HSD), multidimensional analysis of variance (MANOVA) with the Lambda Wilks test, and Mann–Whitney test were applied for testing the significance of differences between trace elements' bioavailability/immobilization in soils, which varied according to the addition of different soil amendments. Statistical significance was accepted at $p \leq 0.05$.

3. Results and Discussion

3.1. Earthworm Survival and Growth in Amended Soils

The biological response of the earthworms significantly depended on the applied soil amendment (Figure 1). The observed mass reduction was diversified from 10% to 100% and was directly related to their mortality. No earthworms survived after adding biosolids to the soil (both GB and BB). After 1 week of the experiment, the earthworms died. We suggest that this effect should be attributed not only to the metal toxicity, but also to the change in living conditions after the application of fresh biosolids. In the case of the other soil amendments, the survival rate (SR) was observed at the level of 40%–93%, but significant changes were only induced by GC and BE (Figure 1). Generally, in all the samples, a reduction in earthworm weight was recorded. The overall decrease was noted in the following order: GC (72%) > BE (33%) > ZC (17%) > CP (15%) > CC (13%) > IO (11%) > RW (10%).

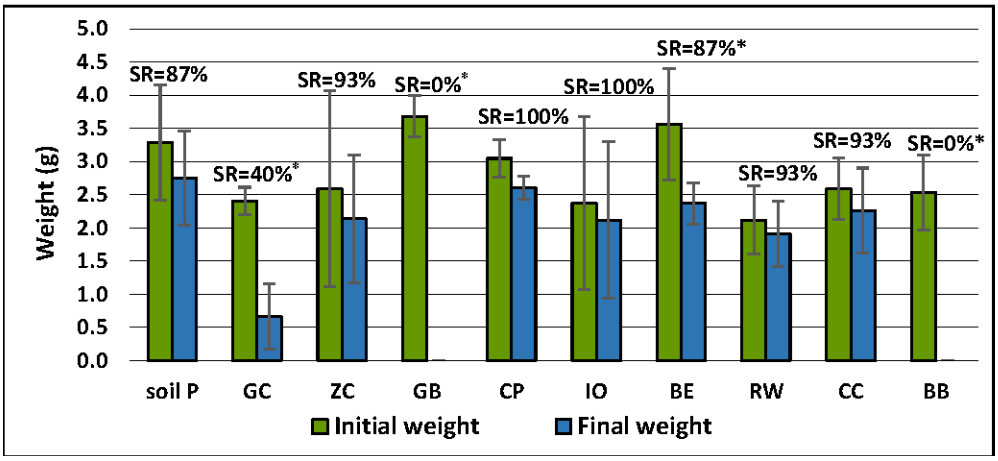

Figure 1. Changes in the weight and survival rate (SR) of earthworms under the influence of soil amend-ments (n = 3). The weight change was expressed in normalization to the number of surviving earthworms * significant differences at $p < 0.05$ in soil between initial and final weight within a given amendment according to the Tukey test; ZC-Zabrze compost, GC-GWDA compost, BB-Bełchatów biosolids, GB-Grabów biosolids, CP-Calcium phosphate, IO-Iron oxide, BE-Bentonite, RW-Rock waste, CC-Limestone.

The great importance of organic amendments for improving soil quality is well known in remediation systems [2,3,14]. However, in our study, some of the amendments adversely affected the survival of the earthworms. One of the potential reasons for the observed mortality due to the application of biosolids is related to the fact that these organic amendments contained additional amounts of TE (Table 1) that could have been easily assimilated by the earthworms. Moreover, the application of biosolids to contaminated soils may increase the bioavailability of metals due to the high amount of destabilized (dissolved) organic matter, which facilitates metal mobility in soil [3,13–15]. According to Tandy et al. [15], compost or biosolids may additionally remobilize some elements that are present in highly recalcitrant fractions (residual Fe-oxide and Al oxides). The negative effect of biosolids on earthworms was also observed by other authors in uncontaminated soils [21]. Combined with the fact that the composts tested in our study did not result in such a mortality of earthworms despite applying similar amounts of trace elements to the soil, this might suggest other causes of earthworm death in biosolid-treated soils. The level of mortality could have been related to the transformation of exogenous organic matter, which is less stabilized in biosolids than in biosolid-compost. The more intense mineralization of organic matter in the soil with the addition of fresh biosolids can cause strong oxygen deficiency immediately after its mixing with soils, resulting in the death of earthworms. This is only a hypothesis, however; it needs to be tested in detail in separate biotests.

The other commonly applied soil amendments, such as phosphates, iron oxides, calcium carbonate, and clay-rich materials did not affect the survival rate, causing only a slight decrease in the total earthworm weight. These amendments did not bring substantial amounts of potentially toxic trace metals or organic substances to the soil; therefore, their effect on the earthworms was apparently less complex than in case of the biosolids. The effect of these amendments on the earthworms' weight was probably related to the alteration in the TE toxicity due to the amendments. The metals were subjected to immobilization processes after the introduction of these materials to soils, comprising ion exchange, adsorption, occlusion, surface precipitation, and co-precipitation processes.

As summarized by Lemtiri et al. [12], many studies assessing the toxicology of metals to earthworms are available in the literature. Metals were mostly added to soils artificially as solutions in these studies. The data on metal toxicity in contaminated soils are scarce and difficult to interpret due to multiple contaminations, the diverse ages of contamination, and the effects of the soil properties on metal availability. There are studies [8,28,30,36,45,46] explaining the mechanisms of earthworms' survival under stress conditions, which are supported by efficient innate immune mechanisms based on the cellular activities of coelomycetes and humoral immune proteins.

Moreover, the response of earthworms to TE concentration is reported to be species-dependent and, therefore, their response to the accumulation of metals in soil is thought to be diverse. For most species, in general, metals are toxic, and negatively affect their population density and reproduction [2,24,47]. Certain earthworm species, such as *Eisenia fetida*, *Eisenia andrei*, and *E. veneta*, are sensitive to Cd, Zn, Pb, or other chemicals, and can be used as bio-indicators of soil contamination [7,8,12,13,21,24,28,29,48]. Therefore, it is very likely that the toxicity of TE to *E. veneta* was observed in our study, and none of the tested soil amendments were able to fully alleviate it.

3.2. Effect of Applied Amendments on Trace-Element Accumulation in Earthworms

The accumulation of various trace elements in earthworm tissue has been evaluated using biota-soil accumulation factor (BSAF) according to the literature data [8,12,13,21,24,28] and the formula presented in Materials and Methods Section 2.2.2. The results indicated a variation in the metal accumulation by earthworms caused by the type of element and the type of applied soil amendment (Table 2).

Table 2. TE accumulation and biota-soil accumulation factor (BSAF) values in the control and the amended soils (n = 3).

	TE Concentrations in Earthworm Tissue (mg kg^{-1})					
	Zn_{ET}	Pb_{ET}	Cd_{ET}	Cu_{ET}	Ni_{ET}	Cr_{ET}
Soil UP	114.39 ± 28.19	10.45 ± 9.76	10.11 ± 1.45	11.28 ± 1.2	1.12 ± 0.02	0.3 ± 0.04
Soil P	290.3 ± 31.7	408.5 ± 105.8	112.5 ± 25.8	16.0 ± 4.1	0.9 ± 0.3	0.6 ± 0.1
GC	267.8 ± 131.7	311.7 ± 219.5	50.8 ± 9.3	24.8 ± 27.3	1.6 ± 0.7	1.4 ± 0.1
ZC	367.9 ± 109.8	335.9 ± 64.0	48.3 ± 11.9	18.9 ± 2.2	1.8 ± 1.5	2.5 ± 3.0
GB	-	-	-	-	-	-
CP	394.6 ± 338.7	351.8 ± 177.8	66.10.53 ±	18.2 ± 6.7	1.3 ± 1.3	1.4 ± 1.1
IO	358.6 ± 149.5	226.4 ± 75.4	70.5 ± 19.3	17.3 ± 1.1	0.8 ± 0.4	0.7 ± 0.5
BE	336.4 ± 161.6	332.6 ± 90.8	82.7 ± 13.3	20.5 ± 24.3	0.9 ± 0.5	0.8 ± 0.9
RW	246.7 ± 50.9	382.1 ± 50.1	93.8 ± 29.0	19.2 ± 4.1	1.0 ± 0.1	0.3 ± 0.1
CC	270.7 ± 22.2	332.0 ± 24.9	94.3 ± 29.9	20.5 ± 3.4	0.7 ± 0.1	0.1 ± 0.1
BB	-	-	-	-	-	-
	BSAF Values					
	Zn_{BSAF}	Pb_{BSAF}	Cd_{BSAF}	Cu_{BSAF}	Ni_{BSAF}	Cr_{BSAF}
Soil P	0.07 ± 0.01a	0.19 ± 0.05a	0.77 ± 0.18a	0.55 ± 0.14a	0.07 ± 0.03a	0.05 ± 0.01b
GC	0.07 ± 0.02a	0.14 ± 0.10ab	0.35 ± 0.06c	0.66 ± 0.95a	0.13 ± 0.06a	0.13 ± 0.01a
ZC	0.09 ± 0.03a	0.15 ± 0.03ab	0.33 ± 0.08c	0.66 ± 0.08a	0.15 ± 0.13a	0.23 ± 0.20abc
GB	-	-	-	-	-	-
CP	0.10 ± 0.07a	0.16 ± 0.08ab	0.45 ± 0.07bc	0.63 ± 0.23a	0.11 ± 0.11a	0.13 ± 0.10abc
IO	0.09 ± 0.04a	0.10 ± 0.03b	0.48 ± 0.13bc	0.60 ± 0.04a	0.07 ± 0.03a	0.06 ± 0.04b
BE	0.08 ± 0.04a	0.15 ± 0.04ab	0.57 ± 0.09ab	0.71 ± 0.84a	0.07 ± 0.04a	0.07 ± 0.06abc
RW	0.06 ± 0.01a	0.17 ± 0.02ab	0.64 ± 0.20ab	0.67 ± 0.14a	0.08 ± 0.01a	0.03 ± 0.01c
CC	0.07 ± 0.01a	0.15 ± 0.01ab	0.65 ± 0.20ab	0.71 ± 0.12a	0.06 ± 0.01a	0.01 ± 0.01c
BB	-	-	-	-	-	-

The different letters indicate significant differences in accumulation of particular elements across soil amendments ($p < 0.05$) using Tukey test; ZC—Zabrze compost, GC—GWDA compost, BB—Bełchatów biosolids, GB—Grabów biosolids, CP—calcium phosphate, IO—iron oxide, BE—bentonite, RW—rock waste, CC—limestone.

Generally, the earthworms living in soil P absorbed metals proportionally to their concentration level in the soil, whereas the applied soil amendments significantly modified their bioavailability. The BSAF index indicated that the greatest susceptibility to accumulation in unchanged soil was exhibited by the Cd (Cd: 0.77), followed by the other measured elements (Cu: 0.55, Pb: 0.19, Zn: 0.07, Ni: 0.07, and Cr: 0.05). The results imply that the availability of metals depends on the chemical properties of the applied soil amendments, which significantly affect the risk of uptake of the harmful elements from the soil environment. After four weeks of experiments, the TE accumulation factor for these elements was observed in the amended soils within the following ranges: Cd: 0.35–0.65, Cu: 0.66–0.71, Pb: 0.10–0.17, Zn: 0.07–0.10, Ni: 0.07–0.15, and Cr: 0.01–0.23. The BSAF for the Cd decreased to the greatest extent after the addition of ZC (by 57%), GC (55%), CP (41%), and IO (37%), while it was statistically insignificant after the addition of BE (26%), RW (17%), and CC (16%). A similarly positive effect was noted for the Pb after the addition of IO (45% decrease). The other amendments somewhat lowered the Pb concentration values, but the average values were not statistically different. The amendments did not significantly affect the Zn and Cu accumulation in the earthworms; however, the numbers observed suggested that in soils treated with organic amendments, Zn and Cu might tend to accumulate more than in untreated soil. The increased accumulation of Zn and Cr does not diminish the beneficial effect of composts, since a major risk for the trophic chain is related to Cd. Furthermore, greater Zn supply reduces the accumulation of Cd in animal bodies [49]. Moreover, no pronounced effects of amendments were observed for the Ni bioaccumulation. The Cr bioaccumulation was reduced by the RW and CC amendments, while it was slightly stimulated by the ZC, GC, and CP. It is worth noting, however, that these differences were observed at low levels of both Cr concentration and BSAF. Therefore, for a full picture of the amendments' effects on the Cr bioaccumulation in earthworms, biotests shall also be performed using Cr-rich soils.

The results showed that earthworms accumulate various contaminants in their bodies to different extents, and this process can be modified by soil amendments. According to Batier et al. [26], Nannoni et al. [50], and Nannoni et al. [45], the major metal-bearing phases in the soils surrounding Pb- and Zn-smelters are pyromorphite for Pb and Fe-oxyhydroxides and smectites for Zn. The very low solubility of pyromorphite can explain the relatively low bioaccumulation of the Pb in the earthworms in the long-term contaminated soil used in our study. At the same time, a long-term aging process may also modify the potential toxicity and bioavailability of metals in soils. In general, ageing processes decrease the negative environmental effects of metals in soils over time, and long processes of natural stabilization decrease the concentration of residual pollution, although residual soil pollution problems may be still detected.

The assimilation of metals by earthworms occurs through two pathways: (i) absorption following dermal contact and the ingestion of organic matter and soil particles, and (ii) adsorption through the gut tissues [8,13,29,31,45,50]. Because of the diversity of ecological characteristics and feeding habits of different species, the relative importance of dermal contact versus gut adsorption may be diversified [8,13,31]. The main mechanisms that regulate the accumulation of metal in earthworm tissue are related to the ability of these organisms to eliminate excess metals. Wang et al. [13] showed that for essential metals, such as Cu and Zn, a fast initial uptake was followed by equilibrium after a few days of exposure, caused by physiological control and the possible excretion of these elements.

The varied metal uptake by earthworms may additionally be associated with ecological species diversity and their ability to adapt to unfavorable environmental conditions [21,25,29]. Earthworm species are generally classified into three broad ecological groups. These include epigeic earthworms, which both feed and burrow in the litter layers on the surfaces of soils, endogeic earthworms, which primarily inhabit the organic-matter-rich top-soil profile [13,21,25,29], and anecic earthworms, which move both vertically and horizontally in soil and form burrows in the lower compartments of the soil profile. Wang et al. [13], Suthar et al. [29], Wen et al. [45], Hodson [47], and Verma et al. [32] observed species-specific metal accumulation patterns. They found that endogeic earthworms accumulated more TE in their tissue by feeding on soil variously contaminated with metal-bearing mineral phases than epigeic earthworms. Species-specific ingestion behavior, burrowing habits, and earthworm niche structures have a major impact on TE accumulation in earthworm tissues [8,13,21,29,31,51].

3.3. Influence of Amendments and Earthworm Activity on Soil pH

It is well established that soil pH is a key factor affecting adsorption–desorption behaviors and, hence, the bioavailability of TE to earthworms [10,13]. In our study, this parameter was also analyzed to estimate the dependence of soil pH on the earthworms' activity and the soil amendments (Figure 2). The earthworms' activity did not significantly affect the soil pH. In contrast to earthworms, the additives caused a significant pH shift over the course of the experiment. The organic amendments lowered the soil pH_{H2O} drastically, from 8.0 to 6.9–7.1, in the biosolid (BB, GB)- and GC-treated soils, while the ZC caused only a small pH decrease (0.2 unit). This difference can be explained by the pH of the amendments: the pH of ZC was alkaline (7.4), whereas the other organic amendments were slightly acidic (5.3 and 6.1 for the GC and GB, respectively) or neutral (6.7 for BB) (Table 1). The observed pH decrease explains the greater Cd and Zn solubility in the GC-, GB-, and BB-amended soils (Figures 3 and 4). The bentonite induced a small increase in the soil pH, since its initial pH was 9.0. The calcium carbonate did not have a strong effect on the soil pH, which was related to the initially alkaline soil pH.

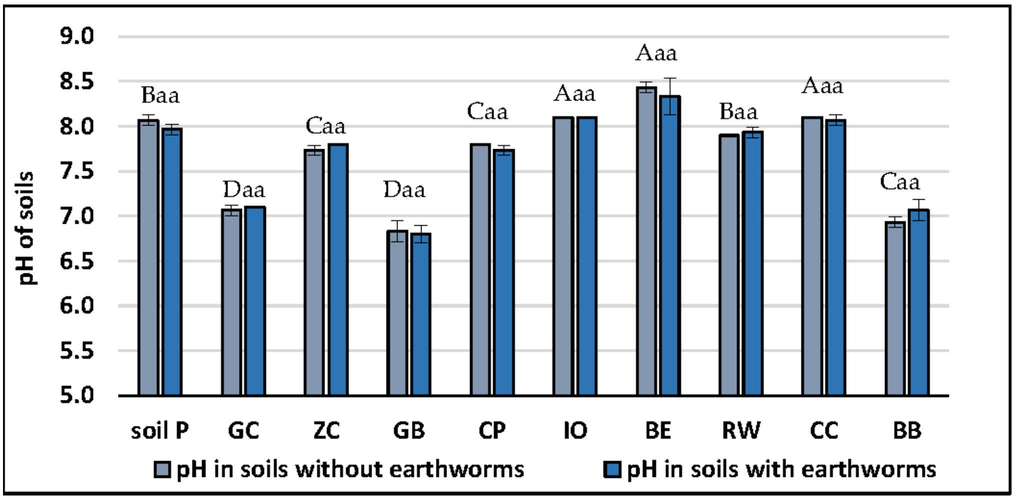

Figure 2. Influence of amendment application and earthworm activity on soil pH. Different lower case letters within the same type of amendment application indicate significant differences between pH in soils with and without earthworms, while capital letters indicate significant differences between applied amendments (ZC-Zabrze compost, GC-GWDA compost, BB-Bełchatów biosolids, GB-Grabów biosolids, CP-Calcium phosphate, IO-Iron oxide, BE-Bentonite, RW-Rock waste, CC-Limestone; ANOVA, Mann-Whitney test, $p < 0.05$).

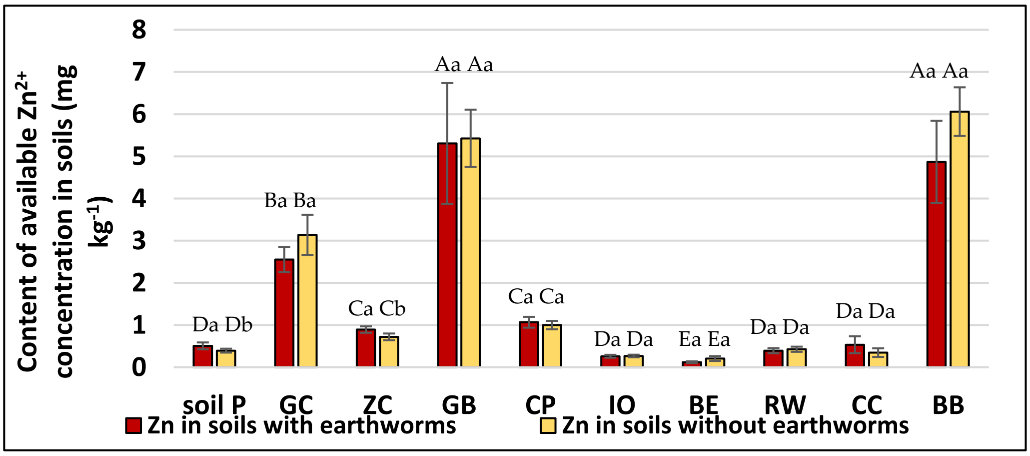

Figure 3. Content of extractable Zn in soils affected by soil amendments and earthworms activity. Different lower case letters within the same type of amendment application indicate significant differences between individual Zn concentration in soils with and without earthworms, while capital letters indicate significant differences between applied amendments (ZC-Zabrze compost, GC-GWDA compost, BB-Bełchatów biosolids, GB-Grabów biosolids, CP-Calcium phosphate, IO- Iron oxide, BE-Bentonite, RW-Rock waste, CC-Limestone; ANOVA, Mann-Whitney test, $p < 0.05$).

The pH of the contaminated soils is usually the most important chemical property governing trace-metal solubility through a range of sorption, precipitation, or occlusion processes [52]. The metal sorption related to particular soil constituents is also pH-dependent—organic soil constituents can bind some metals strongly, even at acidic pH, by forming chelates with humic acids, whereas the sorption capability of iron oxides becomes stronger at a pH of around 7 [53].

Basta et al. [52] observed that earthworm activity increased soil pH due to the excretion of calcium compounds into the environment by calciferous glands. It must be pointed out that all types of earthworm, with or without calciferous glands, may increase soil pH due to their alkaline urine [47]. In our study, metal toxicity might have affected these processes.

However, the lack of effect of the earthworms on the soil pH was mainly related to the fact that the initial soil pH was alkaline.

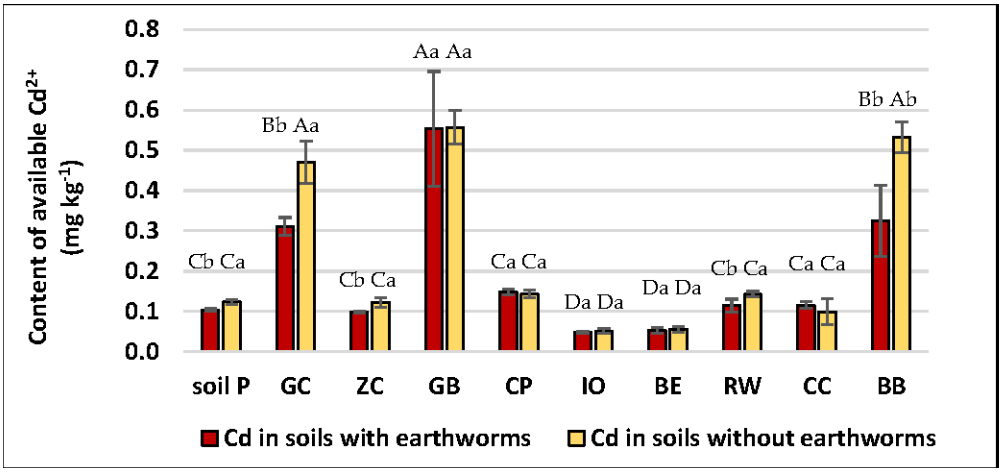

Figure 4. Content of extractable Cd in soils affected by soil amendments and earthworm activity. Different lower case letters within the same type of amendment application indicate significant differences between individual Cd concentration in soils with and without earthworms, while capital letters indicate significant differences between applied amendments (ZC-Zabrze compost, GC-GWDA compost, BB-Bełchatów biosolids, GB-Grabów biosolids, CP-Calcium phosphate, IO-Iron oxide, BE-Bentonite, RW-Rock waste, CC-Limestone; ANOVA, Mann-Whitney test, $p < 0.05$).

3.4. Solubility of Trace Elements in Soils Driven by Applied Amendments and Earthworm Activity

The extractability of metals in the 0.01M $Ca(NO_3)_2$ was dependent on the applied soil amendments and, in some cases, on the activity of the earthworms. Among all the analyzed TE, only the Zn and Cd were characterized by higher contents of the extractable fraction, while the other elements (Pb, Cu, Ni, and Cr) were below the detection limit (0.081 mg kg^{-1}, 0.050 mg kg^{-1}, 0.032 mg kg^{-1}, and 0.099 mg kg^{-1}, respectively for the Pb, Cu, Ni, and Cr). Generally, the content of extractable Cd (Figure 2) in the non-amended soil was about six times lower than that of the Zn (Figure 3), which was partly related to the magnitude of their total contents in the soil. On the other hand, the extractable Zn fraction accounted for 0.01% to 0.015% of their total content, while the Cd accounted for 0.03% to 0.38%. A higher proportion of soluble cadmium resulted from their constant hydrolysis, indicating the potential mobility and solubility of the metals in the soil. The value of this parameter was much higher for the Cd (pK = 10.1) than for the Zn (pK = 7.7) or the other analyzed TE.

The content of the extractable Cd and Zn was strongly driven by the properties of the applied amendments. The organic amendments, such as the composts (ZC and GC), and biosolids (BB and GB) significantly increased both the Zn and the Cd extractability, while the calcium phosphate induced a slight increase in the soluble Zn. By contrast, the bentonite and iron oxide reduced the solubility of the Cd and the bentonite reduced the solubility of the Zn (Figures 2 and 3). The mobilization of metals, such as Zn and Cd, in contaminated soils treated with organic amendments was observed previously and can be attributed to pH shifts or the release of large amounts of humic acids from biosolids [42,54,55]. However, the observed increased Cd solubility in the compost-treated soils was not reflected by a raised bioaccumulation of Cd in the earthworm tissues. In fact, the compost reduced the BSAF value for the Cd. This discrepancy indicates that the 0.01M calcium nitrate extraction used might simulate Cd mobility, but not necessarily Cd bioaccumulation, in earthworms.

The earthworms' activity also significantly influenced the availability of the metals; however, the observed effects were not the same for both metals and differed between the combinations. The earthworms reduced the Cd extractability only in certain cases—in the soils treated with the GC, ZC, RW, and BB, but also in the control without treatments (soil P), which suggests that this process was influenced by the interaction between the amendment properties and the activity of the earthworms. For the Zn, we observed the opposite processes—the earthworms slightly mobilized the Zn in the control and ZC-amended soil. When soil is ingested and passes through the earthworm gut, the pH value of the soil is adjusted to neutral pH via the excretion of calcium carbonate [32,48]. The calciferous glands in the worms, including *Eisenia veneta*, secrete amorphous calcium carbonate, which crystallizes in the gut to calcite and aragonite [24,40,46,47,53]. The excretion of these minerals into the soil constitutes additional sorbent for metals and reduces their mobility in soils [18,48]. This process could have contributed to the reduced Cd solubility in the compost-treated soils.

The bioavailability of TE is related to their chemical forms in the soils. Several fractions or compartments of the soil act as reservoirs of available metals. The literature shows that although they are not perfect, tests based on the determination of most available metal pools give better answers for evaluating the toxicity of soils than total metal concentrations [10,11,13,25,29]. According to Violante and Pigna [11], the bioavailability of TE theoretically decreases gradually with time from the available (mobile) form to the residual (immobilized) form. Among the different forms, the exchangeable form is connected to the highest risk for living organisms because it features the greatest mobility. However, in natural ecosystems, the occurrence of these processes can be disturbed [11]. A number of factors related to soil properties, such as pH, organic-matter content, porosity, the surface charge of the soil components, as well as TE characteristics related to the residence time, solubility, reactivity, or constant of hydrolysis, may affect the sorption behavior of the trace elements in soils [11,13,21,25]. Additionally, some soil amendments can significantly affect the durability and availability of metals in soils by influencing their physicochemical properties.

4. Conclusions

A vast body of literature focuses on the short-term testing of the impact of soil amendment on plant growth or trace-metal solubility. The effect of amendments on the accumulation of potentially toxic trace elements in soil organisms is usually overlooked, despite its strong implications for the transfer of contaminants to the trophic chain. We tested a range of amendments commonly used in phytostabilization studies and practice.

No earthworms survived the 4-week period after adding municipal biosolids to the soil, indicating the toxicity of fresh biosolids to these organisms. This fact reveals the limitations of testing biosolids in such short-term bioassays or/and real stress conditions shortly after the application of fresh biosolids to soils. This issue requires further studies involving the dynamics of soils' biological responses to the application of biosolids. The observed decrease in earthworm weight might indicate that no amendments fully alleviated the toxicity of the metals in this heavily contaminated soil from a post-industrial region.

Cadmium easily transfers from soil and accumulates in earthworm tissues. Our study confirms that this element creates the highest risk for the trophic chain in soils affected by the Zn and Pb smelting industry. Cadmium accumulated in the earthworms at 112 mg per kg of earthworm tissue in a soil containing 146 mg of Cd per kg. For predators, eating earthworms from this type of soil results in the absorption of almost the same dose of cadmium as eating soil.

The effectiveness of composts in the phytostabilization of metal-contaminated soils is well known. In our study, applying composts as single treatments reduced the pH and stimulated an increase in the solubility of cadmium and zinc. This study confirms that in contaminated ecosystems, compost must be accompanied by liming to counteract soil pH shift and prevent the mobilization of metals.

Despite the soil pH decrease, the significant potential of compost to reduce the accumulation of cadmium in earthworms was documented. A significant reduction in cadmium bioaccumulation was also observed after the addition of iron oxide and calcium phosphate. The potential increase in the accumulation of Zn after the compost amendment is not necessarily a negative development from the perspective of the trophic chain. In general, increases in the Zn supply reduce the accumulation of Cd in animal bodies. This study provides valuable practical knowledge on the short-term biological effects of a range of soil amendments in metal-contaminated soils. Further studies shall involve testing combinations of amendments and longer-term experiments, also conducted under field conditions.

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