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ORIGINAL PAPER

Application of a vermiremediation process-based system using *Eisenia fetida* for treatment of highly polluted soils with heavy metals

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Abstract
The objective of this research was to detect the presence of heavy metals (HMs) in soils polluted with sludge from an industrial wastewater treatment plant and then use Eisenia fetida earthworms to reduce the contamination levels in the soil. The sludge was added to the soil at varying rates of 0, 10, 20, 30, 40, and 50 ton/ha. To examine the impact of organic amendment, 12 *Eisenia fetida* earthworms were added to all samples for every 500 g of the contaminated soil. After 42 days, the overall concentration and categorization of HMs in the soil, along with the changes in weight and mortality of the earthworms, were determined. The addition of sludge to the soil resulted in an elevation of HM levels, including copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), and cadmium (Cd). Notably, with the exception of Cd, the concentration of HMs decreased after the 42-day period following the introduction of earthworms. The highest absorption of metals by the earthworms occurred when 20 ton/ha of sludge was applied. This suggests that the earthworm species *Eisenia fetida* possesses the capability to bioaccumulate Zn and Cd, as indicated by a bioaccumulation factor exceeding 1 for these metals. In conclusion, the utilization of earthworms in this study not only resulted in the storage of HMs within the worms' bodies but also contributed to the improvement of the physicochemical properties of the soil.

Keywords Heavy metals · Earthworm · Wastewater · Bioaccumulation · Sewage sludge

Introduction

Human activities have led to the widespread contamination of soils with heavy metals (HMs) across the globe (Aslani et al. 2021; Koyuncu 2022; Wang et al. 2024). The use of sludge from wastewater treatment as a soil fertilizer results in environmental pollution with HMs such as zinc (Zn), mercury (Hg), arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), and radioactive materials (Su and Critics 2014; Yu et al. 2022). As an organic waste, wastewater sludge can be a valuable source of macro and micro elements (Karagianni et al. 2024). However, sludge and wastewater also possess unfavorable chemical properties. Wastewater sludge contains organic pollutants, HMs, and pathogens. The accumulation of HMs in soil can contaminate food chains and pose irreversible risks to human health (Eid et al. 2022). In a study, it was reported that the availability of HMs went up dramatically with raising sludge content in soil (Jatav et al. 2022). Hence, to minimize soil contamination, determining the optimal quantity of sewage sludge for agricultural use is crucial. The presence of HMs in soil is affected by multiple factors, such as the chemical composition of the sewage sludge, application techniques, soil characteristics, plant varieties, weather conditions, and the inherent chemical properties of the metals (Singh et al. 2020; Zaragüeta et al. 2023).

Several methods have been suggested to purify soils that are polluted with HMs and to avoid the spread of contamination to other sources, such as water. Some of these methods include excavation and earth-filling, landfilling, acid cleaning and soil washing to cleanse contaminated areas (Sethi et al. 2023). Many of the conventional methods for

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soil remediation are impractical due to their high costs or the lack of facilities in developing countries. However, HM stabilization offers a relatively simple and cost-effective alternative that can be implemented in various areas. This method involves the use of a stabilizing agent to convert accessible and washable HMs (free and exchangeable ions) into less soluble forms, such as stable mineral structures, deposition with iron oxides, aluminum, manganese, carbonates, phosphates, and sulfide compounds. These transformed forms can persist in the soil for an extended period, reducing the risk of contamination to the food chain and surface and groundwater resources (Babu et al. 2021). Thus, focusing on the impact of organic and inorganic modifiers on the fixation of heavy metals in soil can be an effective method for preventing the spread of pollutants. Earthworms have been identified as biological agents that could influence the mobility and availability of HMs (Das and Paul 2023), depending on earthworm species, soil type and HM type (Sizmur et al. 2011a, b; Sizmur et al. 2011a, b). It has been demonstrated that earthworm activity in soil can change properties such as pH, organic carbon content, and the activities of other soil organisms, which in turn can affect the availability and distribution of Cd and other HMs (Ge et al. 2023). Rorat et al. examined the capability of *Dendrobaena veneta* earthworms to consume sewage sludge as a source of organic waste. Their observations indicated that appropriate amounts of sewage sludge could serve as a beneficial food source for soil organisms, including earthworms. However, when sewage sludge was used improperly and in large quantities, the earthworms began to die (Žaltauskaitė et al. 2022). Moreover, Sizmur et al. used Lumbricus terrestris earthworms to study the movement of HMs in soil. Their findings showed that soil containing earthworms had higher concentrations of Cu and Pb due to the degradation of organic matter (OM) by the earthworms, which released these metals. The authors also noted that Pb and Cu were more sensitive to changes in OM, while Zn was more sensitive to pH changes. They concluded that an increase in pH due to earthworm activity caused the mobility of Zn to decline (Sizmur et al. 2011a, b). Also, Singh and Kalamdhad employed *Eisenia Fetida* earthworms in soils that were polluted with HMs. Based on the findings, this type of earthworm is efficient in decreasing the bioavailability of metals such as Zn, Ni, Pb, and Cd. They also showed that the earthworm activity reduced the exchange fraction of Zn, Ni and Cu and the residual of these metals increased. Moreover, they stated that calcium excreted by the earthworm intestine increased the soil pH (Singh and Kalamdhad 2013). In a recent study, it was found that the use of rice husk biochar and earthworms together can speed up the process of passivating HMs in industrial sludge during vermicomposting (Wang et al. 2022). Hence, the objective of this research was to investigate the impact of organic modifiers, such as earthworms (*Eisenia fetida*), on the immobilization of HMs in soil contaminated with sewage sludge. Furthermore, the kind of HMs present in the soil was identified and the extent to which the earthworms could modify their behavior and availability within the soil–plant system was tested.

Materials and methods

Used sludge and soil

The sludge was obtained from the wastewater treatment plant of Bu Ali Industrial Town, located in Hamedan, Iran. The treatment process was complete-mix aerated lagoon that purifies the wastewater of different industries like food, textile, metal, electrical and electronics, mineral, cellulose and chemical (flow rate: 900 m^3/d on the average). Furthermore, the required soil was from the farmlands located around Bu-Ali Sina University. Next, the prepared sewage sludge and soil were air-dried and sieved. First, a 2-mm mesh was used to measure soil texture, electrical conductivity (EC), pH, cation exchange capacity, and HM concentration. Then, a 0.5-mm mesh was used to measure OM and calcium carbonate.

Sample preparation

Five samples containing various values of sewage sludge (0 (S0), 10 (S10), 20 (S20), 30 (S30), 40 (S40), and 50 (S50) ton/ha) with three repetitions and a total of 18 containers were prepared for the study. The soil and sludge were well mixed in all samples and poured into plastic containers. The containers were left unattended for 42 days to stabilize the sludge in the soil, during which the moisture of the containers was monitored. The modification started after this period. *Eisenia fetida* earthworms were used to examine organic modifiers. Firstly, the earthworms were weighed and placed in a plastic container containing 500 g of the soil, 12 earthworms and the dishes were kept in an incubator. During the experiment, the survival and weight of the earthworms were monitored weekly. Moreover, some holes were made on the walls and lids of the containers and soil moisture content was always fixed at the level of field capacity. After 42 days, the earthworms were collected from the soil, weighed, and put in a petri dish for 42 h for intestinal emptying. Next, the worms were dried in oven at 105 °C for 3 h to chemically decompose the worm's body tissues. Soil sampling was done before and after using the modifier. The samples were air-dried and sieved using a 2-mm mesh to measure soil texture, pH, EC, cationic exchange capacity, and HMs concentration and then and a 0.5-mm mesh sieve to determine OM and carbonate calcium.

Determination of physical and chemical characteristics of the soil

Soil texture was measured via a hydrometer method on the basis of the Stokes' law (Olarte et al. 2023). Soil pH in 1: 5 soil/water ratio was determined using a Metrohm 744 pH-Meter (Thomas and Sparks 1996). Soil EC was determined in 1: 5 soil-to-water ratio through a 712 mAh EC apparatus and converted to EC at 25 °C (Corwin and Yemoto 2017). Equivalent calcium carbonate was determined by NaOH reverse titration method (Sims 1996). Soil OM was measured by means of the Walkley–black method (Rowell 2014). Soil cation exchange capacity was detected through the Bauer method (Rowell 2014). Total concentrations of soil HMs were extracted using 4 N HNO_3. Fifteen mL of acid was added to 2 g of the samples and placed at 80 °C for 12 h in bain-marie. After being smoothed, the extract was analyzed for HMs concentration using an atomic absorption spectrophotometer (AAS) (AA-220 Varian, U.S.A). Moreover, the Sposito et al. procedure was used to examine HMs distribution in the soil (Sposito et al. 1982).

Determination of the sewage sludge characteristics

The pH and EC of sewage sludge were measured at a sludge-to-water ratio of 1–5. The percentage of sludge organic carbon was determined by the incineration method (540 °C for 6 h) (Singh and Agrawal 2010).

Analysis of earthworms

The Katz and Jenniss method was employed to investigate the decomposition of HMs in earthworm tissue (Katz and Jenniss 1983). After the experiment, the earthworms were separated from the soil and dried in an oven at 105 °C for 3 h. Then, 0.1 g of each sample was weighed and poured into flasks, followed by the addition of 10 ml of 55% HNO_2 to the containers containing the earthworms, which were kept for 12 h. The samples were then incubated for 2 h at 40–60 °C and heated again for 1 h at 120–130 °C. After cooling, 1 mL of 70% HCl was added and the samples were warmed for 1 h at a temperature of 120–130 °C. After cooling again, 5 mL of distilled water was added and the samples were heated to 120–130 °C until the white steam evaporated. Finally, the samples were cooled and the bioaccumulation factor (BAF) in earthworms was calculated (Cortet et al. 1999):

$$BAF = \frac{\text{heavy metal concentration in earthworm}}{\text{total heavy metal concentration in soil}} \tag{1}$$

This factor is typically compared to 1. If the concentration of HMs in the earthworm's body tissue is greater than that in the bed soil, the BAF is greater than 1. Conversely, if the concentration of HMs in the bed soil is greater than that in the earthworm's body tissue, the BAF is less than 1(Reddy and Pattnaik 2009). In this study, the earthworms were manually counted weekly. The weight and survival of them were also recorded weekly.

Statistical analysis

The data were analyzed by means of SAS software. Also, a completely randomized design factorial experiment was utilized to study the impact of sewage sludge on the accumulation of HMs and the effect of organic modifiers (earthworms).

Results and discussion

Physicochemical characteristics of the control soil, wastewater sludge, and wastewater sludge treated soil

Table 1 presents the physicochemical properties of the soil (control) and sewage sludge. The control was not spiked with HMs and the concentration of HMs did not exceed the standard limits. The content of Zn in the sewage sludge was higher than the standard limit of 2800 mg/kg, with a value of 4440 mg/kg. The concentrations of Cu, Ni, Cd, and Pb in the sewage sludge were lower than the standard limit. Table 1 presents the standard limits for HMs in sewage sludge according to the USEPA503 and its standard limits based on soil pH. Table 2 provides observations of the physicochemical properties of the soils treated with sewage sludge and control soils. The statistical analysis showed that the increase of sewage concentration in soil dramatically reduced reduced soil pH and increased EC, CEC, OM, and the percentage of carbonate calcium in soil ($P < 0.01$). Increased use of sewage sludge in soil resulted in a significant increase in total Zn and Pb levels ($P < 0.01$) in all treatments compared to the control, whereas the increases in Cu, Ni, and Cd levels were not significant (Tables 1 and 2).

Impact of earthworm on soil properties and total HM concentrations

The findings of the analysis of variance for the interaction of soils contaminated with the sewage sludge and treated with earthworm have been shown in Table 3. Moreover, the findings from the mean comparisons of the earthworm's effects on chemical properties such as pH, EC, CEC, OM, $CaCO_3$, and total HM concentrations in soil contaminated with various amounts of sewage sludge have been presented in Table 4.

Table 1 The physiochemical properties of the soil and wastewater sludge used and standards limits of HMs

Parameters	Soil	Sludge	Standard limit USEPA503	The standard range of heavy metals in sludge based on soil pH	
				6.5 < pH	6.5 > pH
pH	7.6 ± 0.02	7.3 ± 0.02	–	–	–
EC (ds.m^{-1})	0.2 ± 0.04	1.7 ± 0.01	–	–	–
O.M (%)	1.7 ± 0.06	24 ± 1.2	–	–	–
CEC (cmol^{+}.kg^{-1})	22.1 ± 1.05	–	–	–	–
$CaCO_3$	1.5 ± 0.23	–	–	–	–
Sand	50.8 ± 2.1	–	–	–	–
Silt	29.8 ± 1.01	–	–	–	–
Clay	19.3 ± 0.8	–	–	–	–
Total Zn (mg kg^{-1})	67.5 ± 2.3	4440 ± 5.1	2800	1000	500
Total Cu (mg kg^{-1})	32 ± 2.9	405 ± 3.2	1500	500	250
Total Ni (mg kg^{-1})	63 ± 2.4	99.5 ± 1.9	150	200	100
Total Cd (mg kg^{-1})	2 ± 0.7	5.2 ± 0.01	39	20	5
Total Pb (mg kg^{-1})	48.8 ± 2.2	124.6 ± 1.2	300	1000	300

pH

It was found that incorporating earthworms as organic modifiers into sewage sludge-contaminated soil significantly increased pH in all soil samples compared to those without earthworms ($P < 0.01$) (Fig. 1a). The greatest increase in pH due to the presence of earthworms occurred in the 30 ton/ha sewage sludge treatment, where the pH rose from 7.4 to 7.6. The smallest change was observed in the 10 ton/ha sewage sludge treatment. Additionally, earthworms raised the soil pH in the control (S0) sample. These findings are consistent with those of Singh and Kalamdhad, who also reported a significant increase in soil pH as a result of earthworm activity (Singh and Kalamdhad 2013). Due to the activity and movement of the earthworms, soil particles are ingested and enter their digestive tract. In the earthworm's intestine, various enzymes and calciferous secretions are produced. Consequently, the materials excreted from the earthworm's intestine increase the soil pH because of their carbonate content (Brinza et al. 2013). According to Udovic and Lestan, an elevation in soil pH in HM-contaminated soil was observed when *Eisenia Fetida* earthworms were present (Udovic and Lestan 2007). Also, Vig et. al. stated that soil pH increases by adding earthworm (Vig et al. 2011). They asserted that the rise in soil pH attributed to the presence of earthworms was due to heightened activity among soil microorganisms. They proposed that earthworms facilitated an increase in the soil microbial community, leading to the release of organic nitrogen and subsequent ammonium release into the soil. As ammonium dissolved in water, it contributed to an elevation in soil pH. However, it is important to note that the impact of earthworms on soil pH and HM availability can vary significantly, depending on factors such as soil type and prevailing conditions (Wen et al. 2004). Adding wastewater sludge to the soil prevents an increase in pH and thus reduces worm activity, as evidenced by the control treatment (without sewage sludge) having the highest pH. Except for the application of 30 ton/ha of sewage sludge, no substantial differences were observed in soil pH values when earthworms were present. For soil treated with 30 ton/ha of wastewater sludge, the pH increased by 0.01 units compared to soil treated with 50 ton/ha of wastewater sludge when earthworms were present.

EC

The presence of earthworms in soil contaminated with various levels of sewage sludge led to a decrease in the soil's electrical conductivity (EC) compared to conditions without earthworms, indicating significant changes ($P < 0.05$). Although the use of sewage sludge increased EC, the presence of earthworms reduced the EC in all treatments except for the control sample (Fig. 1b). The treatment of 10 ton/ha of the sewage sludge resulted in the lowest reduction in soil EC, with a decrease of 1.0 dS m^{-1}, while the treatment of 50 ton/ha sewage sludge showed the highest reduction, with a decrease of 0.3 dS m^{-1}. Interestingly, although the presence of earthworms led to a decrease in soil EC, the treatment with 50 ton/ha of the sewage sludge exhibited the highest EC value. This increase in EC can be attributed to the mineral content present in sewage sludge. However, the presence of earthworms caused solute depletion through soil movement and particle ingestion, resulting in a decrease in EC. The results indicated a significant effect of earthworms on soil EC in soils containing sewage sludge, as well as a significant difference in EC among different sewage sludge

Table 2 The physiochemical properties of the soil contaminated with wastewater sludge

Sample (t ha^{-1})	pH	EC (ds m^{-1})	CEC (cmol^{+} kg^{-1})	O.M (%)	$CaCO_3$ (%)	TZn (mg kg^{-1})	TCu (mg kg^{-1})	TNi (mg kg^{-1})	TCd (mg kg^{-1})	TPb (mg kg^{-1})
Control	7.6 ± 0.02^{a}	0.2 ± 0.04^{f}	22.1 ± 1.05^{d}	1.7 ± 0.06^{d}	1.5 ± 0.01^{e}	66.8 ± 2.3^{d}	28.8 ± 2.9^{a}	63.0 ± 2.4^{b}	3.3 ± 0.7^{a}	171.8 ± 2.2^{b}
10	7.5 ± 0.02^{b}	0.4 ± 0.06^{e}	31.0 ± 2.9^{c}	1.8 ± 0.02^{cd}	2.0 ± 0.01^{d}	72.7 ± 6.1^{cd}	32.5 ± 4.2^{a}	92.0 ± 5.7^{ab}	3.3 ± 0.08^{a}	177.0 ± 2.9^{a}
20	7.4 ± 0.01^{b}	0.6 ± 0.05^{d}	33.2 ± 3.0^{c}	1.8 ± 0.02^{c}	3.0 ± 0.02^{a}	84.9 ± 21.1^{bcd}	37.1 ± 8.4^{a}	110.0 ± 19.6^{ab}	3.5 ± 0.6^{a}	192.0 ± 28.6^{a}
30	7.4 ± 0.01^{c}	0.7 ± 0.02^{c}	36.0 ± 3.8^{b}	2.0 ± 0.02^{bc}	2.5 ± 0.02^{bc}	94.0 ± 3.7^{abc}	42.0 ± 12.7^{a}	116.2 ± 47^{a}	3.6 ± 0.3^{a}	195.5 ± 25.6^{a}
40	7.4 ± 0.03^{c}	0.8 ± 0.04^{b}	38.1 ± 4.1^{ab}	2.1 ± 0.01^{ab}	2.8 ± 0.01^{ab}	98.2 ± 15.1^{ab}	44.5 ± 17.8^{a}	118.0 ± 15.2^{a}	3.9 ± 1.1^{a}	195.8 ± 17.2^{a}
50	7.3 ± 0.02^{d}	1.0 ± 0.06^{a}	40.4 ± 4.7^{a}	2.3 ± 0.02^{a}	2.4 ± 0.01^{c}	108.3 ± 8.5^{a}	46.9 ± 2.8^{a}	130.1 ± 37^{a}	3.9 ± 0.06^{a}	204.7 ± 29.6^{a}

The comparison of the means was based on the Duncan test. And, a, b, c and d show the significant difference at 1% level. The values in column 1 show the amount of sludge added to the soil. Also, in the table control is S0. The control sample does not contain sludge (characteristics are reported in Table 1). And, all samples do not contain earthworms

application rates. The treatment with 40 and 50 ton/ha of the sewage sludge had the highest EC value of 0.7 (dS m^{-1}), while the control treatment had the lowest value of 0.2 (dS m^{-1}).

Organic matter

The application of various quantities of sewage sludge increased the soil's organic matter (OM) content. However, the presence of earthworms led to a decrease in OM compared to soil without earthworms, despite the sewage sludge contamination. The activity of earthworms, including their feeding on soil OM and sewage sludge, contributed to the reduction of OM content. These findings align with similar studies conducted by Vig et al., as well as Hait and Tare (Hait and Tare 2011; Vig et al. 2011). When OM moves along the earthworm's intestine, some of it is decomposed, while the remainder is released in solution (Bakar et al. 2011). Singh and Kalamdhad observed a decrease in soil OM content when earthworms were present (Singh and Kalamdhad 2013). They stated that the decrease in soil OM content was likely due to a reduction in carbon content. The presence of earthworms led to the consumption of soil OM as they fed on it during their growth, resulting in a decline in the overall soil OM content.

CEC

As seen in Table 3, the simultaneous effect of earthworms and various quantities of sewage sludge on soil exchange capacity was significant ($P < 0.01$). While different amounts of sewage sludge increased the exchangeable cations, their use in conjunction with earthworms reduced them compared to scenarios without earthworms (Fig. 1c). The activity of earthworms, including the ingestion of soil particles, induces changes in soil physicochemical properties (Bronick and Lal 2005).

Calcium carbonate

The interplay between earthworms and varying levels of sewage sludge exerted a notable impact on calcium carbonate content ($P < 0.05$). Augmenting the application of sewage sludge led to elevated calcium carbonate content, and the presence of earthworms in soils contaminated with different quantities of sewage sludge significantly boosted calcium carbonate levels. Edwards and Bohlen proposed that earthworm activity, including their calcium excretion into the soil, contributes to the accumulation of calcium carbonate (Edwards and Bohlen 1996). Gago-Duport et al. showed the effect of earthworm secreted calcium carbonate on the mobility of Zn (Gago-Duport et al. 2008). Calcium-rich exudates from earthworms not only enhance soil

Table 3 Analysis of variance of mean squares of the earthworm effect on the soil contaminated with wastewater sludge

Change sources	Degrees of freedom	pH	EC	O.M	CEC	$CaCO_3$
Earthworm	1	0.1**	0.06*	1.94^{ns}	863.18**	3.30*
Wastewater sludge	5	0.05**	0.39*	0.17^{ns}	90.19**	1.62*
Earthworm*sludge	5	0.01**	0.01*	0.04^{ns}	41.19**	0.05*
Error	24	0.002	0.03	0.02	2.20	0.01

The comparison of the means was based on the Duncan test. And, ** shows the significant level at 0.01, * shows the significant level at 0.05 and ns shows the lack of difference

calcium levels but also influence the mobility of various elements. Canti and Piearce concluded that carbonate calcium secreted by earthworms not only raises soil pH but also enhances Zn absorption on carbonate surfaces (Canti and Piearce 2003). Lee et al., in their study on calcium carbonate secreted by earthworms, noted that the granules produced by the worms consist primarily of calcite but also contain ventrite and amorphous calcium carbonate (Lee et al. 2008). In the current study, among the samples treated with sewage sludge, the highest calcium carbonate content in the soil was observed in the treatment receiving 20 ton/ha of sewage sludge (3.8%), while the lowest was found in the control soil (2.2%) (Fig. 1d).

HM concentration

Table 5 presents the statistical findings regarding the interaction effects of sewage sludge-contaminated soil used as a fertilizer on the overall concentration of HMs. Additionally, Table 4 displays the results of changes in the total concentration of HMs resulting from the utilization of soils. The use of earthworms as organic modifiers led to a reduction in the total concentration of HMs. The interaction between earthworms and various amounts of sewage sludge resulted in a decrease in the total levels of Cu, Zn, Pb, Ni, and Cd. However, it is important to note that the reduction was statistically significant only for Pb, while it was not significant for the other HMs (as indicated in Table 5). Other researchers have also reported a decrease in HM concentrations in contaminated soil when earthworms are used as a remediation approach (Jain et al. 2004; Suthar 2009; Singh and Kalamdhad 2013). Jain et al. claimed that earthworms with their activity in soil and physiological metabolisms could reduce the availability of HMs (Jain et al. 2004); they expressed that *E. foetida* has the ability to accumulate toxic HMs and possesses a metabolic system capable of reducing hexavalent chromium (Cr(VI)) to trivalent chromium (Cr(III)). Furthermore, Hobbelen et al. reported that the concentrations of copper (Cu) and Zn decreased after a period of 54 days upon introducing earthworms to the contaminated soil (Hobbelen et al. 2006); this may be attributed to an increased energy demand for the regulation and detoxification of HMs by the earthworms. A review article concluded that earthworm activities enhance the ability of plants to accumulate biomass, extractable metals, pore-water contents, and overall metal uptake (Blouin et al. 2013). Eventually, earthworms excrete substances such as humic substances, thereby increasing the activity of other soil microorganisms (Blouin et al. 2013). According to Suthar et al., the utilization of *Eisenia fetida* in soil contaminated with sewage sludge resulted in a notable reduction in the concentrations of Cd, Cr, Cu, and Pb (Suthar et al. 2014). They claimed that this decrease was likely because of the accumulation of HMs in the earthworm body. In the current research, a decrease in the concentrations of the mentioned HMs: Cd, Cr, Cu, and Pb was observed across all treatment samples as well as the control sample. However, the presence of earthworms caused an increase in the total amounts of Cu by 1 unit, Pb by 0.4%, and Ni by 1.3 units in the soil compared to the scenario where no earthworms were utilized.

Absorption of HMs in earthworm body

Tissue decomposition of earthworms, before and after the worm was added to the soil contaminated with the sludge, was used to compare the observations obtained. Additionally, the findings from the chemical analysis of the worm's body tissue have been presented in Table 6. The results indicate that when exposed to different levels of sewage sludge, earthworms were capable of accumulating HMs within their body tissues. Consequently, this led to a temporary reduction in the concentration of HMs. The increase in the concentration of HMs and their accumulation in the body tissues of earthworms is due to the consumption of soil and the absorption of HMs in the body tissues of earthworms. Consequently, this process leads to a reduction in the concentration of HMs in the soil. Indeed, when the content of HMs is not high, earthworms are better able to absorb them. Earthworms exhibited the highest uptake for all HMs except Cd at 20 ton/ha. The maximum absorption for Cd was related to the 30 ton/ha treatment. It is worth noting that, at 40 and 50 ton/ha samples, the amount of absorption was low which may be because of the toxicity of high content of Cd. Although sewage sludge, as a pollutant containing

Table 4 Effect of earthworm on the chemical properties and total content of heavy metals in the soil contaminated with the wastewater sludge

Sample (t ha^{-1})	pH	EC (ds m^{-1})	CEC (cmol^{+} kg^{-1})	O.M (%)	$CaCO_3$ (%)	TZn (mg kg^{-1})	(mg kg^{-1})	TNi (mg kg^{-1})	TCd (mg kg^{-1})	TPb (mg kg^{-1})
S0	7.7 ± 0.03^{a}	0.2 ± 0.002^{d}	21.7 ± 0.7^{b}	1.6 ± 0.03^{a}	2.2 ± 0.04^{e}	62.2 ± 1.6^{b}	29.9 ± 2.6^{a}	88.0 ± 9.7^{a}	2.5 ± 0.04^{b}	170.2 ± 9.1^{a}
S10	7.5 ± 0.05^{c}	0.3 ± 0.06^{c}	22.9 ± 1.7^{ab}	1.4 ± 0.03^{a}	2.1 ± 0.06^{d}	78.5 ± 10.5^{ab}	30.2 ± 4.2^{a}	88.4 ± 0.6^{a}	3.0 ± 0.1^{ab}	176.8 ± 9.5^{a}
S20	7.5 ± 0.05^{c}	0.6 ± 0.03^{b}	23.6 ± 2.4^{ab}	1.3 ± 0.2^{a}	3.8 ± 0.05^{a}	79.2 ± 8.5^{ab}	30.9 ± 8.1^{a}	92.2 ± 21.2^{a}	3.1 ± 0.4^{ab}	178.6 ± 9.4^{a}
S30	7.6 ± 0.04^{b}	0.6 ± 0.08^{ab}	24.0 ± 0.2^{ab}	1.4 ± 0.2^{a}	3.0 ± 0.09^{c}	81.5 ± 10.9^{ab}	33.3 ± 6.6^{a}	99.1 ± 19.2^{a}	3.2 ± 0.4^{ab}	182.9 ± 13.1^{a}
S40	7.5 ± 0.08^{c}	0.7 ± 0.09^{a}	24.6 ± 2.2^{ab}	1.6 ± 0.1^{a}	3.1 ± 0.01^{b}	83.6 ± 12.2^{ab}	41.5 ± 15.6^{a}	114.8 ± 38.1^{a}	3.6 ± 0.5^{a}	193.3 ± 31.1^{a}
S50	7.5 ± 0.04^{c}	0.7 ± 0.03^{a}	25.2 ± 1.1^{a}	1.7 ± 0.3^{a}	3.0 ± 0.06^{c}	88.6 ± 5.2^{a}	44.9 ± 17.2^{a}	121.0 ± 45^{a}	3.6 ± 0.6^{a}	200.6 ± 21.1^{a}

The comparison of the means was based on the Duncan test. And, a, b, c show the significant difference at 1% level. The values in column 1 show the amount of sludge added to the soil. Also, S0 is a soil sample without wastewater sludge and with 12 earthworms per 500 g of soil

HMs, may have a negative impact on the soil microbial communities, the average sewage sludge used in the soil (20 and 30 ton/ha) represented a suitable value. It served as a food supply for the earthworms. In addition to feeding on organic matter in wastewater sludge, the earthworms exhibited higher activity in the presence of 20 ton/ha sludge, thereby exerting a greater effect on the soil, which led to a reduction in HMs due to their increased activity. The increase in earthworm activity in the 20 ton/ha sample and its decrease in samples with higher concentrations may be attributed to the presence of toxic substances and HMs that hinder earthworm activity in samples exceeding 20 ton/ha. Earthworms can store HMs from soil in their body tissues. Thus, these organisms serve as indicators for evaluating the bioavailability of HMs in contaminated soil (Lapinski and Rosciszewska 2008). According to Wang et al., the accumulation of metals in the bodies of worms is considered a unique phenomenon. Each metal has its own specific mechanism and process for absorption or decomposition throughout the worm's intestine that is very complicated to understand (Wang et al. 2013). Table 7 gives a breakdown of earthworm survival. As can be seen, the highest mortality rate was for the control sample. Considering the rate of mortality of the earthworms during the test period, a conclusion can be drawn that the addition of sludge resulted in a decrease in the mortality rate. Although the sludge contained high quantities of HMs, the earthworms could intake HMs and survive as they consume wastewater as a food source (Bajsa et al. 2004). Accordingly, in control samples without any sludge addition, the earthworms experienced higher mortality rate. According to Brinza et al., who examined the impact of earthworms on the immobilization of Zn, they found that the survival rate of earthworms decreased in soils contaminated with Zn; they also reported that the survival and tolerance of the earthworms are attributed to the species of earthworms and type and content of HMs (Brinza et al. 2013). Sizmur et al. stated that the weight of the earthworms may affect their mortality rate; they observed that in soils, to which earthworms having lower weight in the beginning of the test period were added, more counts of the earthworms died (Sizmur et al. 2011a, b; Sizmur et al. 2011a, b). Furthermore, Vig et al. explained that the population number of the earthworms declined slowly after 30 days in polluted soil with HMs; the researchers suggested that the mortality of the earthworms was attributed to the lack of food supply in the soil, as no additional food was added to it (Vig et al. 2011).

Bioaccumulation factor

The results for bioaccumulation coefficient have been indicated in Table 8. Bioaccumulation factor shows the value of metals accumulated in earthworm body. The biological factor of *Eisenia fetida* was in control and contaminated values

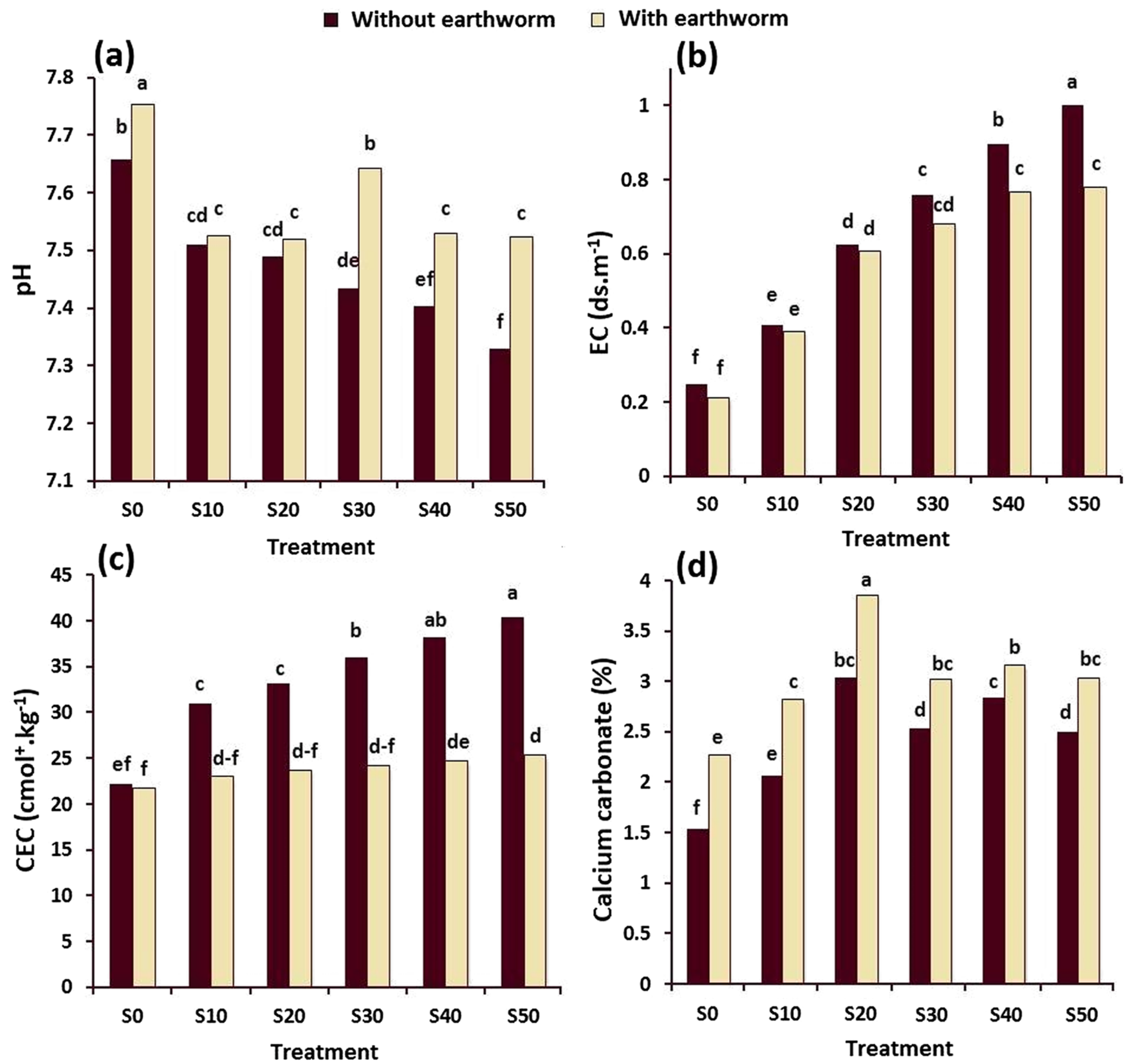

Fig. 1 Changes of pH **a** EC **b**, CEC **c**, and $CaCO_3$ **d** in the soil contaminated with different concentrations of the wastewater sludge and in the presence and absence of earthworm

Table 5 Analysis of variance of mean squares of the interaction between earthworm different concentrations of wastewater sludge

Change sources	Degrees of freedom	Zn	Cu	Ni	Cd	Pb
Earthworm	1	654.8^{ns}	111.3^{ns}	1.4^{ns}	165.4^{ns}	1966.3**
Wastewater sludge	5	865.2^{ns}	256.4^{ns}	0.6^{ns}	1959.4^{ns}	6880.1**
Earthworm*sludge	5	122.0^{ns}	17.7^{ns}	0.0^{ns}	370.6^{ns}	4132.8**
Error	24	129.3^{ns}	105.5^{ns}	0.3	733.2	444.4

The comparison of the means was based on the Duncan test. And, **shows the significant level at 0.01, *shows the significant level at 0.0 and ns shows the lack of difference

of sewage sludge for two metals: Zn and Cd were higher than 1. However, except for 50 ton/ha of sewage sludge in Zn metal with a value of 0.9, in the control treatment with no sewage sludge the biological factor content was higher than one for Pb. Nonetheless, for the copper and nickel in the control treatment and for the copper and nickel and Pbmetals for all treatments, the biological factor was less than 1. The biological factor is highly dependent on earthworm species (Cortet et al. 1999). The results indicated that between *Aporrectodea caliginosa* and *Lumbricus rubellus*, the biological

Table 6 Total concentration of heavy metals in the tissue of earthworm body

Sample (t ha^{-1})	TZn (mg kg^{-1})	TCu (mg kg^{-1})	TNi (mg kg^{-1})	TCd (mg kg^{-1})	TPb (mg kg^{-1})
Control	87.1 ± 1.3^{c}	19.5 ± 1.6^{a}	47.6 ± 0.6^{ab}	8.1 ± 0.4^{ab}	55.0 ± 1.6^{ab}
10	99.0 ± 12.5^{bc}	21.1 ± 3.6^{a}	52.5 ± 4.1^{ab}	8.7 ± 0.3^{ab}	55.4 ± 5.1^{ab}
20	122.7 ± 20.9^{a}	24.4 ± 6.2^{a}	60.2 ± 14.8^{a}	8.4 ± 0.7^{ab}	63.4 ± 7.8^{a}
30	121.2 ± 8.9^{ab}	18.4 ± 0.6^{a}	51.0 ± 4.9^{ab}	9.3 ± 0.5^{a}	48.0 ± 8.3^{b}
40	118.6 ± 8.7^{ab}	19.0 ± 3.0^{a}	47.4 ± 1.6^{ab}	7.7 ± 0.4^{b}	58.6 ± 6.6^{ab}
50	106.1 ± 7.8^{bc}	20.8 ± 0.9^{a}	44.3 ± 2.9^{b}	8.4 ± 0.3^{ab}	57.6 ± 2.7^{ab}

The comparison of the means was based on the Duncan test. And, a, b, c and d show the significant difference at 1% level

Table 7 The survival of earthworm during the treatment (earthworm number per 500 g of soil)

Sample (t ha^{-1})	Early tests	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	End of test
Control	12	12	10	9	7	7	6	5
10	12	12	11	11	10	10	9	9
20	12	12	12	11	10	10	10	9
30	12	12	12	11	10	10	10	10
40	12	12	11	11	10	10	10	9
50	12	12	12	11	11	10	10	10

Table 8 Bioaccumulation factor in earthworm

Sample (t ha^{-1})	Zn	Cu	Ni	Cd	Pb
Control	1.3 ± 0.06^{c}	0.6 ± 0.07^{a}	0.7 ± 0.03^{a}	2.4 ± 0.06^{c}	1.1 ± 0.02^{a}
10	1.3 ± 0.02^{b}	0.6 ± 0.04^{c}	0.5 ± 0.06^{b}	2.6 ± 0.05^{a}	0.3 ± 0.01^{c}
20	1.4 ± 0.05^{a}	0.6 ± 0.04^{b}	0.5 ± 0.05^{c}	2.3 ± 0.06^{d}	0.3 ± 0.02^{b}
30	1.2 ± 0.02^{d}	0.4 ± 0.01^{e}	0.4 ± 0.03^{d}	2.5 ± 0.04^{b}	0.2 ± 0.1^{f}
40	1.2 ± 0.01^{e}	0.4 ± 0.02^{e}	0.4 ± 0.02^{e}	1.9 ± 0.02^{f}	0.2 ± 0.01^{d}
50	0.9 ± 0.1^{f}	0.4 ± 0.02^{d}	0.3 ± 0.01^{f}	2.1 ± 0.02^{e}	0.2 ± 0.04^{e}

The comparison of the means was based on the Duncan test. And, a, b, c and d show the significant difference at 1% level

factor was higher for *Aporrectodea caliginosa* (Dai et al. 2004); they attributed this variation to the differing capacities of various earthworm species to accumulate HMs in their body tissues. In his study, *Eisenia Andrei* earthworm in soil contaminated with mineral pollutants, Lukkari et al., concluded that the bioaccumulation factor for Cr and Pb was less than 1 (Lukkari et al. 2006); this illustrates the lack of accumulation of these two metals in the soil, but this factor was more than 1 for cobalt and nickel. In this study, Zn and Cd in the earthworms were higher than other metals. Moreover, this species of earthworms was able to absorb more Zn and Cd from the soil, increasing their bioavailability by more than 1. Our findings align with those of Morgan et al., who observed that the bioaccumulation factor of earthworms for Cd and Zn was greater than 1 (Morgan and Morgan 1999). Suthar et al. found that the presence of earthworms in HM-contaminated soil, coupled with the mineralization of OM during their activity, resulted in an increased bioavailability of HMs (Suthar et al. 2014). These findings are in line with the results reported by Wang et al., who discovered that the introduction of earthworms to contaminated soil led to the mineralization of OM, which directly influenced the bio-mobility and bioavailability of HMs (Wang et al. 2013). It should be noted that a decline in the earthworm weight was observed during the treatment process (Fig. 2). The highest amount of weight decrease was for the samples of 20 and 30 ton/ha. As mentioned above, there was the highest amount of HM absorption in these two samples, illustrating that the earthworms were more active in them. Although the pollution of soil with wastewater sludge declined the weight of the earthworms, they were capable of intaking more HMs. Since no substrate was added to the soil samples over the process, the only food source for the earthworms was the added sludge, which was rich in OMs. The earthworms in samples: 40 and 50 ton/ha, which had the highest levels of organic materials, exhibited the least weight reduction. The earthworms in sample of 10 ton/ha experienced a regular downward trend in their weight over the process. Weight

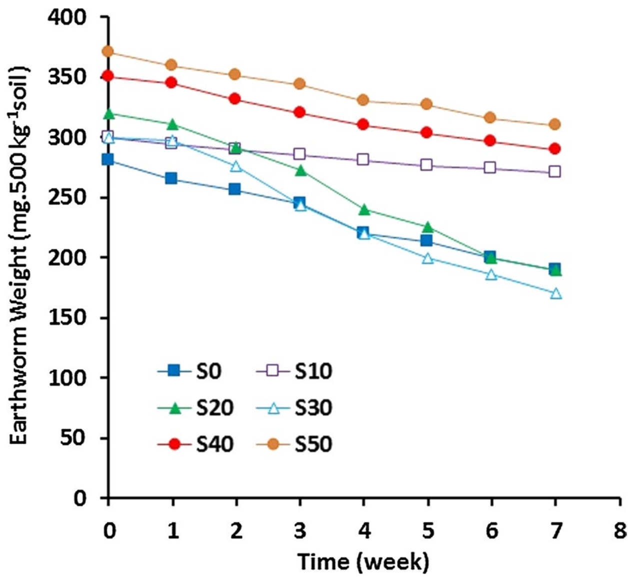

Fig. 2 Decrease of earthworm weight during the treatment period

decrease of the control sample was approximately like that of 20 and 30 ton/ha; this decline can be due to the fact that the earthworms did not have any food source. According to Kaur et al., the weight of earthworms is directly correlated with the availability of food sources and the consumption of degraded substrates (Kaur et al. 2010). Also, Gomez-Brandon et al. concluded that the growth of earthworms is dependent on microbial community and available substrate (Gómez-Brandón et al. 2013). The findings from the study conducted by Singh et al., who examined the impact of manure on the growth of earthworms in HM-polluted soil, revealed that the weight of earthworms that received the highest amount of manure in their environment increased during the middle of the testing period. However, towards the end of the period, a decrease in weight was observed across all the samples. This weight decline was attributed to a decrease in the activity and tolerance of the earthworms (Singh and Kalamdhad 2013).

Conclusion

When wastewater sludge was added to the soil, the total HM content increased, with the highest rise observed at 50 ton/ha. However, the activity of earthworms led to a reduction in the concentrations of Cu, Pb, Ni, and Cd. Earthworms consume wastewater sludge as a food source and consequently store HMs in their tissues, with the highest absorption rate observed at 20 ton/ha of sludge. Despite using the sludge as food, the earthworms' weight decreased. Notably, the highest mortality rate occurred in the control sample, indicating that Eisenia fetida can tolerate HM pollution in soil. Additionally, the bioaccumulation factor for Zn and Cd exceeded 1, demonstrating that this species can effectively bioaccumulate these metals.

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Author contributions Ghasem Azarian: Conceptualization, Analysis and interpretation of data Writing—review & editing. Ghasem Rahimi: Writing—review & editing, Supervision, Writing-review. Kazem Godini: Writing—original draft. Maryam Farhanian: Acquisition of data.

Data availability All the data and material pertinent to this paper are included and have been reviewed by all the authors.

Declarations

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval This article does not contain any studies with human subjects or animals conducted by any of the authors.

Consent to participate All the authors are informed and agree to this study.

Consent for publication All the authors agree to publication in this journal.

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