Bioremediation of Lead and Zinc Contaminated Soils by Compost Worm

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Abstract

Background: This study investigated the bioremediation of lead and zinc in contaminated soils by the compost worm Eisenia Fetida.

Methods: This experimental study was conducted in vitro using 108 samples. The initial concentrations of 50 and 100 mg/kg for zinc and lead respectively as well as 40 mg/kg and 80 mg/kg for the control group were studied. 30 earthworms were used for bioremediation of 500g samples of the polluted soils during 14 and 28 days. Then, Pb and Zn were measured by atomic absorption kit (Varian 240) in the soil and earthworm's tissue.

Results: The mortality rate of earthworms was insignificant statistically, so that it was lower than 20% when exposed to 86 mg/L of lead. Moreover, the removal efficiency of Pb and Zn was higher than 90% in th soil. Initial concentration of Pb and Zn was 3 and 6 mg/kg and the bioaccumulation was 0.16 and 32 µg/g respectively during 14 days, while they were 0.31 and 59 µg/g at the end of 28 days. The removal efficiency of Pb and Zn was increased as the exposure time and concentration of Pb and Zn in the earthworm bodies increased.

Conclusion: As a consequence, the use of earthworms is an appropriate organic and cost-effective method for bioremediation of Pb and Zn significantly. However, the improvement and modification of bioaccumulation in earthworm bodies is an environmental challenge that should be managed.

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Introduction

Due to industrial, development, heavy metal (HM) pollution of soils has been increased worldwide.[1] They may originate from and leak to the soils through anthropogenic resources such as industrialization, mining, smelting, and land application of sewage sludge. Among heavy metals, Pb and Zn are considered as the most hazardous heavy metals/metalloids.[2] The HM pollution of soil is a significant environmental issue because they are stable and highly resistant to degradation process as well as toxicological effect on human health through food chain contamination.[3] Lead accumulates in different organs of the body including the kidney, liver, spleen, nervous system, bone marrow, and adrenocortical glands after it enters the body. Increased lead concentrations in the body result in severe toxicity that leads to death if left untreated. Toxicity occurs if the concentration of zinc in the body exceeds the safe level (2 mg/day).[4] Therefore, they are one of the most challenging issues that should be removed. There are several methods for cleaning of Pb and Zn from the soil. However, traditional methods are mainly costly and rarely compatible with ecosystems as well as destructive to the soil. Therefore, they are not very efficient. Hence, the use of new methods with high efficiency and low cost for remediation of contaminated soils is essential.[5] Remediation of contaminated soils using earthworms appears to be cost-effective and environmentally friendly, especially in low concentration of heavy metals.[6] Thus, the pollutant concentrations in the soil are reduced through bioaccumulation mechanisms in the body of the earthworms. These organisms can accumulate high concentrations of heavy metals in their body.[7] This method helps to decrease pollutants through several mechanisms including their intake and accumulation in the earthworm's body, pollutants degradation in the body by gastrointestinal enzymes such as cytochrome P450, certain degrading microbial flora such as Pseudomonas, Nocardia, and alkaligenesis present in the earthworm. On the other hand, they are the main components of biomass. Moreover, earthworms are the most important food source for other organisms higher in the food chain.[8] Previous studies have shown that the presence of excess heavy metals in the soil leads to increased mortality of the worms.[9] The study of Darling and Thomas (2005) showed that the concentration of soluble lead compounds in earthworm bodies was more than that of low soluble compounds. During their study, Avila et al. (2009) identified that increasing the organic material can reduce the toxicity of heavy metals in the body of earthworms. Irizar et al. (2015) concluded during their study that if the organic material in the soil is low, earthworms are not able to digest the soil and, as a result, the toxicity of heavy metals increases in them, and the mortality and disorder in reproduction rise. Haghparast et al. (2013) have shown that organic material is a source of energy for Eisenia Fetida earthworms and increases the percentage of their survival.[10] Other studies such as Nahmani et al.'s study (2009) demonstrated that earthworms could accumulate heavy metals in their bodies and increase the removal percentage of heavy metals from the soil by increasing the pollutant concentration. Moreover, Suthars et al. (2008) demonstrate that earthworms have potential to accumulate different types of heavy metals in their tissues and can be used as an index of environmental pollution.[8] Li et al. (2010) reported that Eisenia fetida could accumulate lead and zinc in high concentrations in their bodies.[11] Since there has been little attention to bioremediation of metals with low concentration by vermicompost, this study aimed to investigate the removal efficiency of Zn and Pb by Eisenia Fetida.

Materials and methods

This experimental study was conducted in vitro using 108 samples. The earthworm used in this study was from the Lumbricidae family, Eisenia genus, and fetida species that was manually identified in the bovine feces of traditional animal husbandry with reference to the guideline and then transferred to the laboratory. Required soil samples were taken from farmlands at 0 to 30 cm depth and, after drying, with a 2 mm (50 mesh) sieve, they were kept in shadow until the experiment began. The amounts of lead and zinc and certain physicochemical characteristics of the soils were measured according to the routine protocols of Iran's Soil and Water Research Institute.[12] Increase in organic compound included 5% and 9% of the soil weight. After the organic compound was prepared and dried, it was sieved for homogenous mixing Table 1. To prepare contaminated soil, 1 mL and 2 mL of 50 g/L of lead solution and 40 g/L of zinc solution were added homogeneously to 500 g soil-containing pots; therefore, 50 mg/kg and 100 mg/kg concentrations of lead and 40 mg/kg and 80 mg/kg concentrations of zinc were obtained. To add the heavy metal, first the soil and organic compound of each pot were spread on a plastic sheet and the solution was added using a manual sprinkler. Then, 30 earthworms were added to each pot and kept under (25±2 °C) and 70% humidity.[13] To measure the concentrations of lead and zinc in the soil, approximately 4 g of the soil of each pot was taken at completion of each step of the experiment (at 14 and 28-day intervals). After the soil samples were dried and ground, 1 g of each sample was weighed by a digital scale to the nearest 0.0001 g, w digested and extracted using concentrated nitric oxide and chloridric acid, 50% (v/v) on a heater set at 95°C. The samples were filtered using a Whatman filter paper grade 1 and kept in polyethylene containers till measurements.[14] To measure the concentrations of zinc and lead in the earthworm body, the added earthworms were removed from the soil environment at completion of each step of the experiment, washed with water, dried using a handkerchief, and left fasting in a wet filter paper inside a glass plate for 24 hours so that their intestinal contents were emptied. Then, the earthworms were collected, washed again, dried and then left in covered vials. To measure the concentrations of lead and zinc, acid digestion was used. In this method, first the earthworms' tissues were dried in an oven after freezing, and then 1 g of the resulting tissue was weighed and poured into an Erlenmeyer, and then 5 mL of concentrated nitric acid and 1 mL of hydrogen peroxide were added. The resulting solution was heated at 180-220°C to obtain a clear solution. The samples were filtered after cooling.[15] To measure the zinc and lead, an atomic absorption kit (Varian 240) was used. Data analysis was conducted using one sample t-test, analysis of variance, and post hoc tests in SPSS 23 and Design-Expert 7.00; $P<0.05$ was considered as the significance level. To calculate the mortality percentage of the earthworms, the number of destroyed pits was divided by that of all pots.

Results

3.1. The effects of different concentrations of the pollutants and intervals on the mortality percentage of

Table 1: The mortality rate of earthworms

Mortality rate of earthworms	Concentration of Pb (mg/kg)		Concentration of Zn (mg/kg)	
	3	103	46	86
14 days	0	3.7	0	0
28 days	7.4	18.5	7.4	14.8

Table 2: Some physicochemical properties of the soil under test

SP% Saturation percentage	Pb (mg/kg)	Zn (mg/kg)	EC (Ds/m)	pH	T.N.V%	OC%	N%	K (ppm)	P (ppm)	Physical Test			
										Sand%	Silt%	Clay%	Texture
76	3	6	0.79	7.57	13.5	3.18	0.30	960	199	27	47	26	Sandy Loam

the earthworms

The mortality percent of earthworms after 14 and 28 days of exposure to polluted soil is shown in Table 1.

According to Table 2, there was a positive relationship between the mortality rate of earthworms and increase in the concentration of Pb and Zn as well as exposure time. Moreover, exposure to Pb increased the mortality rate of earthworms compared to Zn.

3.2. The effects of concentration, organic compound, and intervals on the removal rate of metals from soil and their accumulation in the earthworms' bodies

The initial lead concentration of 3 mg/kg reached 78.2 mg/kg at a 14-day interval and 58.2 mg/kg at a 28-day interval and decreased to 0.22 mg/kg and 0.42 mg/kg in the soil, respectively. The lead concentration of 53 mg/kg reached 33.35 mg/kg at a 14-day interval and 25.21 mg/kg at a 28-day interval and decreased to 19.65 mg/kg and 27.79 mg/kg in the soil, respectively.

The lead concentration of 103 mg/kg reached 57.02 mg/kg at a 14-day interval and 37.65 mg/kg at a 28-day interval and decreased to 45.98 mg/kg and 65.35 mg/kg in the soil, respectively. The initial zinc concentration of 6 mg/kg reached 5.64 mg/kg at a 14-day interval and 5.31 mg/kg at a 28-day interval and decreased to 0.36 mg/kg and 0.69 mg/kg in the soil, respectively. The zinc concentration of 64 mg/kg reached 29.43 mg/kg at a 14-day interval and 22.19 mg/kg at a 28-day interval and decreased to 16.57 mg/kg and 23.81 mg/kg in the soil, respectively. The zinc concentration of 86 mg/kg reached 50.99 mg/kg at a 14-day interval and 35.58 mg/kg at a 28-day interval and decreased to 35.01 mg/kg and 50.42 mg/kg in the soil, respectively (Table 2).

The initial concentrations of zinc, 6, 46, and 86 mg/kg reached 64.5, 43.29, and 99.50 mg/kg after 14 days and 31.5, 19.22, and 58.35 mg/kg after 28 days, respectively. All values, therefore, declined over time.

In the earthworms' bodies, 0.16 µg/g of lead was accumulated in 3 mg/kg concentration at a 14-day interval and reached 0.32µg/g at a 28-day interval. 54.17 µg/g and 50.25 µg/g of lead were accumulated in 53 mg/kg concentration and 44.76 µg/g and 63.33 µg/g of lead were accumulated in 103 mg/kg concentration at 14 and 28-day intervals, respectively (Table 3).

Lead accumulation, at 53, 3, and 103 mg/kg, reached 0.16, 17.54, and 44.76 µg/kg after a 14-day exposure and 0.32, 25.50 and 66.33 µg/kg after 28-day exposure, respectively, in the earthworms' bodies.

Furthermore, 0. 31µg/g of zinc was accumulated in 6 mg/kg concentration at a 14-day interval that reached 0.59 µg/g at a 28-day interval.15.44 µg/g and 21.88 µg/g of zinc were accumulated in 46 mg/kg concentration and 32.44 µg/g and 47.44 µg/g of zinc were accumulated in 86 mg/kg concentration at 14 and 28-day intervals, respectively (Table 4).

Discussion

The Effects of Initial Concentrations of Zn and Pb on Earthworms' Mortality Rate

The results of this study demonstrated that the earthworms' mortality rate was significantly

Table 3: Removal rates of pollutants from soil in initial concentrations at 14 and 28-day intervals

The amount of lead removal from the soil (mg/kg)	3		53		103	
	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
14 days	0.22±0.06	<0.0001[×]	19.65±3.15	<0.0001***	45.98±3.26	<0.0001*
28 days	0.20±0.05	<0.0001[××]	8.14±0.72	<0.0001****	19.37±0.72	<0.0001**
Zinc removal rate from soil (mg/kg)	**6**		**46**		**86**	
	Mean±SD	**P value**	**Mean±SD**	**P value**	**Mean±SD**	**P value**
14 days	0.36±0.16	<0.0001[e]	16.57±2.01	<0.0001[c]	35.01±2.97	<0.0001[a]
28 days	0.33±0.14	<0.0001[f]	7.24±0.62	<0.0001[d]	15.41±1.17	<0.0001[b]

* Statistical difference the residential lead concentration between 3 and 103 mg/kg after 14 days
** Statistical difference the residential lead concentration between 3 and 103 mg/kg after 28 days
*** Statistical difference the residential lead concentration between 53 and 103 mg/kg after 14 days
**** Statistical difference the residential lead concentration between 53 and 103 mg/kg after 28 days
[×] Statistical difference the residential lead concentration between 3 and 53 mg/kg after 14 days
[××] Statistical difference the residential lead concentration between 3 and 53 mg/kg after 28 days
[a] Statistical difference the residential zinc concentration between 6 and 86 mg/kg after 14 days
[b] Statistical difference the residential zinc concentration between 6 and 86 mg/kg after 28 days
[c] Statistical difference the residential zinc concentration between 46 and 86 mg/kg after 14 days
[d] Statistical difference the residential zinc concentration between 46 and 86 mg/kg after 28 days
[e] Statistical difference the residential zinc concentration between 6 and 46 mg/kg after 14 days
[f] Statistical difference the residential lead concentration between 6 and 46 mg/kg after 28 days

Table 4: Bioaccumulation rates of pollutants in earthworms' bodies in initial concentrations at 14 and 28-day intervals

Lead accumulation rate(μg/g)		**3**		**53**		**103**	
		Mean±SD	**P value**	**Mean±SD**	**P value**	**Mean±SD**	**P value**
Time(day)	14	0.16±0.32	<0.0001[×]	17.54±2.06	<0.0001***	44.76±4.01	<0.0001*
	28	0.32±0.11	<0.0001[××]	25.50±2.82	<0.0001****	63.33±4.79	<0.0001**
Zinc accumulation rate(μg/g)		**6**		**46**		**86**	
		Mean±SD	**P value**	**Mean±SD**	**P value**	**Mean±SD**	**P value**
Time(day)	14	0.31±0.15	<0.0001[e]	15.44±1.99	<0.0001[c]	32.44±3.35	<0.0001[a]
	28	0.59±0.28	<0.0001[f]	21.88±2.20	<0.0001[d]	47.44±4.30	<0.0001[b]

* Statistical difference the bioaccumulation lead concentration between 3 and 103 mg/kg after 14 days
** Statistical difference the bioaccumulation lead concentration between 3 and 103 mg/kg after 28 days
*** Statistical difference the bioaccumulation lead concentration between 53 and 103 mg/kg after 14 days
**** Statistical difference the bioaccumulation lead concentration between 53 and 103 mg/kg after 28 days
[×] Statistical difference the bioaccumulation lead concentration between 6 and 46 mg/kg after 14 days
[××] Statistical difference the bioaccumulation lead concentration between 6 and 46 mg/kg after 28 days
[a] Statistical difference the bioaccumulation zinc concentration between 6 and 86 mg/kg after 14 days
[b] Statistical difference the bioaccumulation zinc concentration between 6 and 86 mg/kg after 28 days
[c] Statistical difference the bioaccumulation zinc concentration between 46 and 86 mg/kg after 14 days
[d] Statistical difference the bioaccumulation zinc concentration between 46 and 86 mg/kg after 28 days
[e] Statistical difference the bioaccumulation zinc concentration between 6 and 46 mg/kg after 14 days
[f] Statistical difference the bioaccumulation lead concentration between 6 and 46 mg/kg after 28 days

correlated with the concentrations of Pb and Zn in the soil. The mortality rate increased over time and the concentrations of Pb and Zn increased; this is consistent with the results reported by Zaltauskaite and Sodienė (2010). In addition, Spurgeon et al. (1995) reported that cadmium, lead, copper, and zinc caused a decrease in the growth and an increase in the mortality of Eisenia fetida earthworms. Moreover, t similar results were observed when the Eisenia fetida were exposed to higher concentration of Cr and Cd.[16] It is obvious that the mortality rate of the earthworms increased due to heavy metals-induced toxicity, the increased concentration of metal ions in the soil as increased exposure time (extended exposure to the ions).[17] Because the longer exposure of earthworms such as Eisenia fetida to Pb suppresses the enzymatic activity as well as the damage of histology and change of metabolism.[18] As a result, the raising concentration of Pb and Zn and exposure period cease the growth of earthworm that reduces the efficiency of removal of Pb and Zn. This result might limit the application of vermicomposting in real scale. Because there are various concentrations of heavy metals in this scale of experiments; therefore, modifying of earthworms or co-composting could be a better choice for bioremediation of heavy metals.

The Effects of the Concentrations and Intervals of Pollutants on Removal Efficiency

In this study, a higher level of Pb and Zn was removed by increasing the concentration of the pollutants; 52.44 and 63.45% (approximately 11% increase) of lead was removed at 53 mg/kg and 103 mg/kg concentrations, respectively, and 51.76 and 58.63% (approximately 7% increase) of zinc was removed at 46 mg/kg and 86 mg/kg concentrations, respectively. These findings are consistent with those of several studies.[19] Moreover, longer exposure of the earthworms to lower concentrations of Pb and Zn caused no substantial change in the removal efficiency. In contrast, increased exposure time of the earthworms to high concentrations of Pb and Zn caused an increase in the removal rate, so that the average removal rates of Pb on 14 and 28-day intervals were 40.86% and 57.94%, respectively, and the average removal rates of zinc at 14 and 28-day intervals were 38.36% and 55.2%, respectively. In other studies, the efficiency removal of Zn, Pb, Cd znd Cu increased after 42 days using Eisenia fetida.[14] The life-cycle parameters such as the exposure level, uptake level, target level, and internal pathway of detoxification were affected by metal contamination.[12-20]Likewise, a similar effect was observed for media conditions, especially pH. It is the most effective parameter on heavy metal contamination and the rate of Zn and Pb extracted from the soil.[17] The results of Davies et al.'s study confirmed these findings. They reported that the removal rate of Pb increased by approximately 43% with increasing Pb concentration from 3000 μg/g to 5000 μg/g and exposure time from 14 days to 28 days. Further removal of pollutants can occur for several reasons such as increased exposure of earthworms and other microorganisms, greater surface evaporation, and higher levels of environmental degradation.[21] As a consequence, the increase of exposure time is an effective parameter on the removal efficiency. As the bioremediation using earthworms is a cost-effective method and increasing the duration of operating can increase the cost of process partially, it is a suitable alternative for bioremediation of heavy metals in developing countries such as Iran.

The Effects of Pollutants' Initial Concentrations and Time on Bioaccumulation

The bioaccumulation rate of the metals increased in the earthworms' bodies, while the initial concentration of metals was increased. The mean bioaccumulation rates of lead in 53 mg/kg and 103 mg/kg concentrations were 25.50 µg/g and 63.33 µg/g, respectively, and those of zinc in 46 mg/kg and 86 mg/kg concentrations were 21.88 µg/g and 47.44 µg/g, respectively. Our findings are consistent with those of some studies.[13-16]

The bioaccumulation rates of the pollutants in the earthworms' bodies increased over time such that Pb bioaccumulation rate in 53 mg/kg and 103 mg/kg concentrations increased from 7.96 µg/g concentration at 14-day interval to 18.57 µg/g concentration at 28-day interval. Zn bioaccumulation rate in 46 mg/kg and 86 mg/kg concentrations increased from 6.44 µg/g concentration at 14-day interval to 15 µg/g concentrations at 28-day interval. Because the removal rate of Pb and Zn did not reach 100% after 28 days of the earthworms' exposure to the polluted soil, the accumulation rates of the studied pollutants in the earthworms' bodies increased over time and with increasing the time of the activity because of the earthworm's activity. Moreover, the presence of co-exposure of earthworm to several pollutants modified the bioaccumulation of heavy metals in its body.[22] In a previous study, the Pb concentrations of earthworms in the Pb exposure groups increased with longer exposure times.[13] However, the accumulation rates of metals are less than their removal rates from soil in some cases because of the mortality of earthworms due to environmental incompatibility or excessive toxicity throughout the bioremediation process. Although the presence of earthworms increased the exchangeable and reducible Pb and Zn fractions and decreased the oxidizable and residual fractions from the soil, the accumulation rates of the metals in question in the earthworms' bodies were higher than their removal from the soil in some cases, which can be due to the high volume of soil compared to the number of the earthworms.[11] Therefore, the bioaccumulation of heavy metal is a concern about bioremediation of heavy metals due to their healthy effect on food chain. Although this study described that lead and zinc removal using vermicomposting (Eisenia fetida) remarkably and it is an available, cost-effective and practical method, the bioaccumulation of heavy metals in the worm's body is a challengeable issue because they remain and accumulate in the soil after death of the worm. However, it is suggested that the body worms should be collected and strained before its biodegradation.

Conclusion

The bioremediation of polluted soils is one of the novel and environmentally friendly approaches that can be applied via different methods. The findings of this study demonstrate that Eisenia fetida is an appropriate species for Iran climate conditions and can live in most regions across this country. This earthworm was found to have optimal function to remove the lead and zinc from the polluted soil via its potential to bioaccumulate the pollutants in their bodies. The current study suggests the use of Eisenia fetida as a whole and environmentally friendly method of removing pollutants from soil.

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