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Full Length Research Paper

Study of heavy metals bioaccumulation in the process of vermicomposting

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The bioaccumulation of heavy metals (Cd, Zn, Ni, Pb and Cr) and the relationship between them was investigated on earthworm (*Eisenia fetida)* physiology during the process of vermicomposting. The soil samples were obtained from Roudehen city in the eastern area of Tehran. *E. fetida* specimens were exposed to a mixture of metal compounds in various concentrations to estimate the rate of heavy metal bioaccumulation. After 14-days of (the results of the experiments showed that earthworms accumulated this elements in 14 days) exposure, the metal accumulation was measured using atomic absorption spectroscopy. Cluster analysis was used to interprete the data. Analysis of the 14-day experimental results revealed a strong relationship between the bioaccumulation of Pb and Cr and between Ni and Cd (correlations 0.8 and 0.63).

Key words: Earthworm, *Eisenia fetida*, vermicomposting, heavy metal, bioaccumulation.

INTRODUCTION

Soil pollution from heavy metal contamination has increased to the point that it endangers human life in some areas, and the reduction and eventual elimination of pollution in these areas is urgently necessary. A variety of physical, chemical and biological techniques have been proposed and implemented to achieve this aim (Pereira and Arruda, 2003). One of the methods is the usage of earthworms to clean up the soil from various pollutants, such as heavy metals, by the process of vermincomposting (Bianchina, 2009; Chhotu and Fulekar, 2009).

Vermicomposting represents an excellent treatment method for contaminated soils, not only for waste reduction but also to recondition the soil. Vermicomposting constitutes a special form of composting in which earthworms metabolize and excrete a mixture of soil and organic matter. In the digestive system of these worms, microorganisms transform organic species (proteins, nucleic acids, fats, carbohydrates, etc.) into more stable products in the process of vermicompost (Bianchina, 2009). In addition, earthworms are able to clean up the soil from various pollutants, and are able to accumulate heavy metals on their bodies from the soil too. The process of vermicomposting is used to treat heavy metal contaminated soil through bioaccumulation and conversion to non-toxic forms (Jain and Singh, 2004).

A promising means of determining acceptable levels of soil pollutants lies in the application of ecotoxicity tests. A series of internationally accepted test protocols for toxicity was developed by the Organization for Economic Cooperation and Development (OECD, 1984). The effects of earthworm (*Eisenia fetida*) activity on soil pH, dissolved organic carbon (DOC), microbial populations, fraction distribution and bioavailability of heavy metals was investigated using pot experiments. Some studies showed that earthworm activity increased the mobility and bioavailability of heavy metals in soils (Wen et al., 2004).

The influence of time on lead toxicity in earthworms was studied by Davies et al. (2003). Results of the experiments showed that uptake of lead are linear with time. Chronic toxicity of nickel in three invertebrates was assessed, for which standard test protocols are available (*E. fetida, Enchytraeus albidus* and *Folsomia Candida*). The presented data can be considered as a step forward in the assessment of the potential risks of nickel in terrestrial environments (Lock et al., 2002). Effect of mineral

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Table 1. Primary amount of metals in the samples (µg/g) (14 days).

Element / Sample number	Cd	Cr	Ni	Pb	Zn
1	0.79	0.07	0.55	3.40	0.80
2	0.80	0.08	0.64	4.50	0.96
3	0.85	0.09	0.95	5.00	1.21
4	0.96	0.10	1.20	6.50	1.80
5	1.00	0.11	1.50	7.00	1.89
6	1.01	0.12	1.85	8.00	1.92
7	1.05	0.13	2.30	9.00	2.00
8	1.10	0.14	2.50	9.50	2.10
9	1.30	0.15	3.00	12.50	2.20
10	1.40	0.16	3.20	13.00	2.30

form on lead toxicity shows that the only parameter sensitive enough to distinguish the toxicities of the three compounds was cocoon (egg) production (Davies et al., 2003).

In this study, *E. fetida* was selected because it can be easily used in the laboratory. In addition, an extensive database on effects of all classes of chemicals on this species is available. The important feature of the OECD worm toxicity tests, in this study is its standard practice to add the contaminant to the soil in aqueous form. The result of previous experiments that calculated toxicities of solid compounds are very similar to toxicities calculated from using solution compounds of chemicals (Davis et al., 2002). One difference between this study and the previous researches is that in this study, the vermibed is cow manure and soil, and during the process of vermin-composting, earthworms accumulate metals on their body. Therefore, bioaccumulation causes deduction in soil metals. The objective of this study was to measure the rate of bioaccumulation of the different concentrations of various metal compounds and their mixture in *E. fetida*.

MATERIALS AND METHODS

Sampling

E. fetida Savigny (Lumbercidea) was selected for this study because it can be easily used in the laboratory. Earthworms were obtained from Agricultural Department of the University. In addition, an extensive database containing the effects of all classes of chemicals on this species is available (Edwards et al., 1992). The most relevant feature of the OECD worm toxicity tests in this study is the standard practice of adding the contaminant to the soil in aqueous form. The tests were performed using mixtures of various metal compounds. In accordance with the OECD standard protocol no. 222, the test was conducted in the laboratory at 25 ± 1°C. The procedure began by combining a mixture of soil and vermicompost with a solution of $ZnSO_4$, $NiSO_4$, $CdNO_3$, $K_2Cr_2O_7$ and $PbNO_3$ in distilled water (Table 1). Four replicates of each exposure level and four control experiments were performed. All replicates contained 100 g (dry weight) sieved cow manure and uncontaminated soil (50/50) from the Roudehen Islamic Azad university campus.

Twenty-four hours prior to the start of each test, the samples were prepared as described. Prior to addition to the soil mixture, earthworms were placed on moist filter paper to purge the culture bedding from their gastrointestinal tracts. Each replicate received 10 earthworms (2 g) at the beginning of the test. The test containers were made of glass and covered by a plastic film to permit gaseous exchange between the substrate and the atmosphere while preventing the worms from escaping. The pH of the substrate was measured before and after the experiment. Every week, 2 g of homogenized cow manure was added to the samples. After 14 days, the earthworms were analyzed for the accumulation of metals using a method adapted from the procedure of EPA.

Digestions

Earthworms were placed on moist filter paper to purge the culture bedding from their gastrointestinal tracts. Then samples were digested. In the first step, 10 ml nitric acid was added to the samples, after cooling, more 5 ml nitric acid was added, then 2 ml hydrogen peroxide was added 5 times. At the end, 2 ml hydrochloric acid was added, shaken and heated in each step. Two control plates for each sample were obtained with OECD guidelines.

Determinations and quality control

The samples were analyzed by atomic absorption spectro-photometer (Varian spectra, AA.200). pH meter (p25 Ecomet) KCl 0.01 M, (ISO, 1998) was used to measure the pH of the substrate before and after the experiment. The samples were dried at 60°C and again at 120°C for 24 h, then moisture content of the vermicompost was determined by the percentage loss in weight

Statistical analysis

The mean of the primary and four replicates of accumulative metals concentration were calculated. Then cluster analysis was used to interpret the results and to calculate similarity coefficients.

RESULTS

The initial pH was approximately 7.30 and did not change significantly over the course of the experiments (6.5 to7.6). The moisture content of the vermicompost reached

Table 2. Accumulative amount of metals (µg/g) (14 days).

Element / Sample number	Cd	Cr	Ni	Pb	Zn	pH
1	0.017	0.0001	0.109	0.95	0. 679	7.2
2	0.014	0.007	0.099	3.53	0.505	6.7
3	0.022	0.024	0.161	3.06	0.615	7.1
4	0.023	0.0001	0.379	1.32	0.556	7.4
5	0.016	0.003	0.064	3.1	0.446	6.9
6	0.018	0.061	0.092	2.26	0.359	7.5
7	0.021	0.003	0.194	1.57	0.321	7.5
8	0.019	0.0001	0.173	1.48	0.185	7.2
9	0.021	0.0001	0.163	1.39	0.285	7.1
10	0.022	0.0001	0.212	1.79	0.302	7.2
Mean ± SE	0.0193±0.0029	0.0098±0.0194	0.1628±0.0918	2.045±0.8918	0.4253±0.1605	7.18±0.2529

Table 3. Similarity matrix.

Parameter	Cd	Cr	Pb	Zn	Ni
Cd	1.000				
Cr	-0.334	1.000			
Pb	-0.336	0.789	1.000		
Zn	-0.413	0.116	-0.133	1.000	
Ni	0.622	-0.075	-0.252	-0.462	1.000

Table 4. The amount of similarity coefficient and t-student statistics.

S	T	S	T	S	T	S	T
-0.334	-1.002						
-0.336	-1.009	0.789	3.632				
-0.413	-1.282	0.116	0.330	-0.133	-0.379		
0.622	2.246	-0.075	-0.212	-0.252	-0.736	-0.462	-1.473

42 to 45%, which approximates the saturation limit (44%). Earthworm mortality was assessed by emptying the test medium into a glass plate, sorting worms from the medium, and testing their reaction to a mechanical stimulus at the head end. Typical mortality rates were about 10%. The amounts of each metal accumulated during the experiments are shown in Tables 1 and 2. In the 14-day experiments, increasing the metal concentration decreased the bioaccumulation of Zn and Pb but increased the bioaccumulation of Cd and Ni. Bioaccumulation of Cr did not significantly change over the course of the experiment. The amount of heavy metals that was added to the samples and accumulated amounts of heavy metals by earthworms are shown in Tables 1 to 4 and Figure 1.

DISCUSSION

The results indicated that earthworm activity increases the mobility and bioavailability of heavy metals in soil (Wen et al., 2004; Sizmur and Hodson, 2009). Concentrated heavy metals in the earthworm's body demonstrate the ability of *E. fetida* to accumulate heavy metals in their body. The uptake of each metal is linear in time (Davies et al., 2003), but in a mixture of metals, the interaction between them is not exactly linear. The initial pH was approximately 7.30 and did not change significantly over the course of the experiments (6.5 to 7.6). This value falls within the range (5.7 to 7.8) commonly observed for this material (Pereira and Arruda, 2003). The moisture content of the vermicompost reached 42 to 45%, which approximates the saturation limit (44%); it is the best range of moisture that previous researches reported (Macki et al., 2009; Azarmi et al., 2008).In the 14-day experiment, cluster analysis revealed that Pb and Cr uptake were strongly related and formed what was termed cluster (A) based on their significant similarity coefficient of 0.80. Ni and Cd uptake resulted in a similarity coefficient of 0.63 and formed cluster (B). Bio-

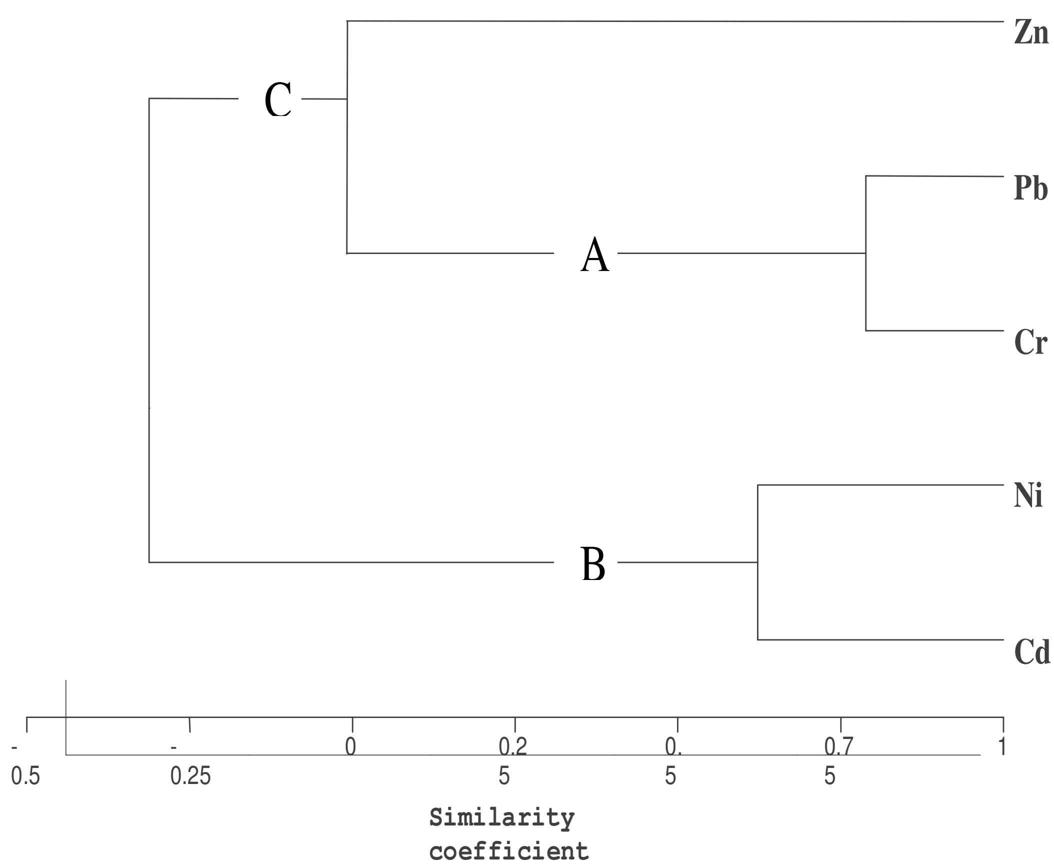

Figure 1. Dendrogram of cluster analysis amongst accumulated metals (14 days).

accumulation of Zn demonstrated an insignificant relationship with other elements and formed cluster (C). The resulting dendrogram for the 14-day experiment contained three distinct clusters, and the similarity coefficient between clusters (A, B and C) was sufficiently low which indicate that there is no relationship between bioaccumulation of Pb and Cr, bioaccumulation of Ni and Cd, and bioaccumulation of Zn. After 14 days, there was a substantial bioaccumulation of Pb, Cr, Ni and Cd by the earthworms, suggesting that vermicomposting is an efficient method for reducing metal pollution in soils.

Soil pH is one of the parameters that affects metal availability (Gupta, 2004), and this was confirmed in this study. In general, however, the use of vermicompost for remediation of environmental contaminants is feasible for which standard test protocols are available. Chronic toxicity of nickel was assessed in three invertebrates (*E. fetida, E. albidus* and *F. candida*), for which standard test protocols are available (Lock et al., 2002). This study considered a step forward in the assessment of the potential risks of nickel in terrestrial environments.

The objectives of this study were to measure the bioaccumulation of various metal compounds in *E. fetida,* and to statistically evaluate the results. This study differs from previous research in that the vermibed is cow manure mixed with the natural soil at the Roudehen campus of Islamic Azad University. During the process of vermicomposting, earthworms accumulate metals internally, resulting in a reduction in metal levels in the soil (Ruiz et al., 2009), which is also reported here. Interaction of the metals mentioned in some other papers (Macki and Samadyar, 2009) is now confirmed by this method too.

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