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Bioaccumulation of Heavy Metals by Iranian Earthworm (Eisenia Fetida) in the Process of Vermicomposting

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Abstract: The possible bioaccumulation and the effect of the concentration of heavy metals such as Cadmium, Zinc, nickel, lead and Chromium (Cd, Zn, Ni, Pb and Cr) on *Eisenia fetida* (Iranian native earthworm) were investigated through vermicomposting. The samples were obtained from agricultural field of Islamic Azad University (IAU), Roudehen branch on the eastern part of Tehran. *Eisenia fetida* was exposed to a mixture of varying concentrations of metal compounds to estimate the rate of bioaccumulation of heavy metals. The bodies of earthworms with the accumulated metals were evaluated within 7, 10, 14, 21 and 28 days after treatment by atomic adsorption spectrometry. Estimation of time, pH and moisture were determined along with the mass concentration of the accumulated heavy metals. The statistical model of the results show that the interaction of metals cause a positive linear relationship between the amounts of added heavy metal such as Ni and Cd and the rate of bioaccumulation, but there was a negative linear relationship between the amount of Pb, Zn, Cr and the rate of body weight of earthworms. In addition, increase in pH had a positive effect, but moisture and the various times of exposure had negative effects on the rate of bioaccumulation of heavy metals.

Key words: Bioaccumulation, Cadmium, Zinc, Nickel, Lead, Chromium, Vermicomposting, *Eisenia fetida*

INTRODUCTION

The increase in soil pollution levels, particularly by heavy metals, has endangered human life. Attempts have been made to reduce and eliminate pollutants from the environment. Physical, chemical and biological methods may be used to achieve this objective.

The availability of some metals must be determined because they are beneficial at low concentrations, but harmful at higher concentration. The availability of metals added with the vermicompost is affected by the components of the vermicompost as well as by the soil characteristics, such as pH and salinity. Earthworms are able to accumulate heavy metals in their bodies from the soil and complex those by other compounds so might be having less toxicity [1]. One promising way of determining acceptable levels of pollutant in soils lies in the application of ecotoxicocity tests. A series of internationally accepted test protocols for toxicity was developed by the Organization for Economic Cooperation and Development [2]. Chronic toxicity of nickel was assessed in Eisenia fetida, by standard test protocols. the presented data can be considered as a step forward in the assessment of the potential risks of nickel in terrestrial environments [3]. It is supposed that the activities of earthworms increase the mobility and bioavailability of heavy metals in soils [4]. The concentration of metals, particularly lead in the worms, was determined at various sampling times. The uptake of lead compound concentrations showed a linear relationship with time [5].

Vermicomposting is one of the best ways to dispose the wastes, not only due to its capacity of reducing the wastes, but also due to its ability to remediate and amend the soil [6]. Vermicomposting constitutes a special form of composting, because it is accomplished when earthworms metabolize and excrete a mixture of soil and organic mater. In the digestive system of these worms, microorganisms are responsible for transforming some organic compounds (proteins, nucleic acids, fats, carbohydrates) into a

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more stable product (vermicompost). In the process of vermicomposting it supposes that earthworms are useful to clean up the soil from various pollutants, such as heavy metals [7].

The only parameter that is sensitive enough to distinguish the toxicities of the mineral compounds is cocoon (egg) production [8]. Standard toxicity tests need to be taking into account the form in which the contaminant is present in the soil to be of environmental relevance [5].

In the present study, Eisenia fetida was selected because it is a native species of Iran and can be easily cultivated in the laboratory conditions. In addition, an extensive database about the effects of all classes of chemicals on this species is available [6]. The important feature of the OECD worm toxicity tests in this research is its standard practice to add the contaminant to the soil in the aqueous form. The result of the previous experiments that calculated toxicities of solid compounds are very similar to toxicities calculated using solution compounds of chemicals [5]. In the present experiment, the vermibed is cow manure and the natural soil of Roudehen region, which is polluted by heavy metal compounds. During the process of vermicomposting, metals get accumulated in the body of earthworms; therefore, bioaccumulation can be used for detecting soil pollution. The objectives of this research were to measure and model the rate of bioaccumulation of the different concentrations of various metal compounds in the mixture on Eisenia fetida and the effects of metal interaction, time, moisture and pH on this rate.

MATERIALS AND METHODS

Vermicompost Preparation: Vermicompost was prepared in the agricultural field of Roudehen Islamic Azad university, (IAU) campus by using cow manure and native earthworm (Eisenia fetida). Initial C/N ratio of the materials used in vermicomposting processes, varies according to its composition, by using cow manure, this value will approximately 20.

Sampling: Tests were carried out using twenty-one different concentrations of various metal compounds in the mixture and the earthworms (Eisenia fetida). According to a standard protocol [2] the test was conducted in the laboratory at 25±1°C. Metal tests were performed by contaminating the mixture of soil and vermicompost by $ZnSO_4$, $NiSO_4$, $CdNO_3$, $K_2Cr_2O_7$, $PbNO_3$ dissolved in distilled water. Four replicates of each exposure levels and four controls were obtained. All replicates contained 100 g (dry weight) sieved cow manure and soil (no contaminated) from (IAU) Roudehen Campus and then they were contaminated by adding various chemical compounds. Each replicate received 10 earthworms at the beginning of the test. Test containers were made of glass and covered by plastic; however, these permitted gaseous exchange between the substrate and the atmosphere while preventing the worms from escaping. Distilled water was added to the samples (50 w/w). Twenty-four hours prior to the start of each test, the samples were prepared and then the earthworms were weighed approximately (0.2–0.4 g) and placed on moist filter paper to depurate culture bedding from their gastrointestinal tracts. Every week 2 g of homogenized cow manure was added to the samples.

Sample Analysis: After 7, 10, 14, 21 and 28 days, the earthworms were analyzed for the accumulation of metals using a method adapted from the procedure of OECD. [2]The samples were analyzed by atomic absorption spectrophotometer (Varian spectra, AA.200). For pH measurements, a pH meter (p25 Ecomet) *KCl* 0.01 M, (ISO, 1998) was used. The pH of the substrate was measured before and after the experiment. The moisture content of the vermicompost was determined by the percentage loss in weight after the sample was dried at 60 °C and at 120 °C for 24 h.

Data Analysis: The rate of accumulation of metals in the worms' bodies and the effect of pH and moisture on it were estimated. A linear model estimated by regression method and surveyed if the statistical tests proved the accuracy of the model. Chi-square, t-student and F-statistics prove the stability of the coefficients and the significance of the model.

RESULTS AND DISCUSSION

Soil-Vermicompost characteristics: The pH value provides valuable information about the decomposition stage of organic matter during the humification process and metal availability is affected by it too. In the present work, the pH and changes significantly over the course of the experiments (5.7 to 7.8) that commonly found in this material [7]. The moisture content of vermicompost reached 42–45% during the process.The mortality was assessed by emptying the test medium onto a glass plate, the worms were sorted from the medium and their reaction

Table 1: The primary amount of metals added to the samples *mg/kg*

	Cd	Cr	Ni	Pb	Zn	T	pH	Mo
1	81	8	64	457	96	14	7.54	43.5
2	83	9	95	525	121	14	7.54	45.0
3	84	9.5	97	527	123	14	7.63	42.2
4	96	10	120	634	179	14	7.58	42.3
5	110	12	186	690	192	14	7.61	39.7
6	111	12.5	188	692	194	14	7.62	42.4
7	112	13	263	921	316	14	7.48	41.6
8	200	20	382	1599	320	14	7.53	42.1
9	202	21	384	1601	322	14	7.6	43.6
10	218	22	418	2044	335	14	7.58	42.5
11	220	22.5	420	2046	337	14	7.47	42.9
12	223	24	430	2297	338	14	7.54	43.8
13	225	24.5	433	2299	340	14	7.61	41.9
14	113	14	280	970	205	7	7.51	43.5
15	157	16	360	1500	250	7	7.48	44.1
16	220	23	430	2308	340	7	7.55	43.8
17	123	15	318	1216	220	10	7.59	44.2
18	79	7	55	343	88	21	7.59	44.1
19	102	11	156	657	182	21	7.62	44.5
20	172	17	360	1635	260	28	7.48	43.7
21	185	18	370	1680	270	28	7.53	42.9

Table 2: The accumulative amount of metals by earthworms, *mg/kg*, pH value and moisture content in each sample and worm data

Element Sample No.	Cd	Cr	Ni	Pb	Zn	pH	Mo	Worm
1	0.0530	0.0001	0.0880	1.2500	0.8300	7.54	43.50	0.93606
2	0.0180	0.0001	0.0840	0.5900	0.6270	7.54	45.00	0.47430
3	0.0180	0.0020	0.0780	2.6900	0.5993	7.63	42.20	1.78518
4	0.0170	0.0001	0.1090	0.9500	0.9476	7.58	42.00	0.75711
5	0.0140	0.0070	0.0990	3.5300	0.5050	7.61	39.70	2.14512
6	0.0220	0.0240	0.1610	3.0600	0.6145	7.62	42.40	1.89540
7	0.0230	0.0001	0.3790	1.3200	0.5566	7.48	41.60	0.91930
8	0.0160	0.0030	0.0640	3.1000	0.4461	7.53	42.10	2.03386
9	0.0180	0.0610	0.0920	2.2600	0.3596	7.60	43.60	1.49182
10	0.0210	0.0030	0.1940	1.5700	0.3212	7.58	42.50	1.12032
11	0.0190	0.0001	0.1730	1.4800	0.1846	7.47	42.90	1.03994
12	0.0200	0.0001	0.1630	1.3900	0.2852	7.54	43.80	1.01563
13	0.0200	0.0001	0.2120	1.7900	0.3022	7.61	41.90	1.29889
14	0.0200	0.0230	0.1680	2.1200	0.2166	7.51	43.50	1.35931
15	0.0240	0.0510	0.2390	5.2200	0.3123	7.48	44.10	3.50359
16	0.0230	0.0040	0.2080	1.5200	0.2992	7.55	43.80	1.11547
17	0.0390	0.0820	0.2560	1.2100	0.6463	7.59	44.20	0.89904
18	0.0290	0.0090	0.1140	0.5800	0.7173	7.59	44.10	0.47323
19	0.0001	0.0001	0.1830	0.6800	0.7143	7.62	44.50	0.54632
20	0.0240	0.0030	0.2500	1.0900	0.5877	7.48	43.70	0.83025
21	0.0180	0.0001	0.2530	1.5100	0.6058	7.53	42.90	1.10872

to a mechanical stimulus was tested. Response of the worms to mechanical stimulus was positive and the percentage of mortality was about 10.

Primary amount of metals added to the samples are shown in Table 1 and the accumulative amount of metals are shown in Table 2. The estimated model shows the effects of metals interactions, pH, time and moisture on bioaccumulation of metals in worms.

Estimated Linear Model for the Mixture of Elements:

$$Worm = -7.424 + 0.026Cd - 0.103CrD1 - 0.002Pb + 0.009Ni - 0.009Zn - 0.056T + 2.906PH - 0.294mo + 0.355AR(1) - 0.768AR(2) - 0.979MA(2)$$

Where,
R^2=0.97
F= 28.72.

Worm = the weighted mean of bioaccumulation results;

$$worm = \frac{\sum (primary \quad amounts \times accumulation \quad amounts)}{\sum primary \quad amounts}$$

Cd = the primary amount of Cd.
CrD1= unknown variable multiplied by the primary amount of Cr.

(CrD1 means that there is an unknown factor (dummy variable) D1 (biochemical or biological) in this experiment that interacts with Cr, affecting the accumulation rate of metals in the worm.)

Pb = the primary amount of Pb.
Ni = the primary amount of Ni.
Zn = the primary amount of Zn.
pH = the pH value.
mo = the percent of moisture.
T = the days of experiments.

Bioaccumulation of Metals: The estimated model showed the rate of metal bioaccumulation. In Table 3, R-square is 97%, which means that most of the variations in the dependent variable (body burden of worm) are explained by the independent variables. The F-statistics in the estimated model is significant for all independent variables [because F-statistic is 28.7209 and probability (F-statistic) is 0.000092]. The elasticity of each metal is calculated by multiplying the coefficient of the metal with the mean of the metal divided by the mean of the bioaccumulation of metals in worms.

Table 3: The estimated statistical model

Development Variable: Worm

Method: Least Squares Sample (adjusted): 3 21

Included observation: 19 after adjusting endpoints

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	-7.424963	7.523897	-0.986851	0.3566
Cd	0.026432	0.004411	5.992511	0.0005
CrD1	-0.103818	0.009630	-10.78083	0.0000
Pb	-0.001931	0.000438	-4.405858	0.0031
Ni	0.009213	0.002089	4.409313	0.0031
Zn	-0.009360	0.001956	-4.785927	0.0020
T	-0.056479	0.011082	-5.096351	0.0014
pH	2.906284	0.958414	3.032390	0.0191
Moisture	-0.294251	0.039590	-7.432411	0.0001
AR(1)	0.355328	0.272788	1.302579	0.2339
AR(2)	-0.768680	0.322670	-2.382246	0.0487
MA(2)	-0.979528	0.195918	-4.999671	0.0016
R-squared	0.978323			
S.E. of regression	0.167327			
Mean dependent var.	1.333605			
S.D. dependent var.	0.708733			
F-statistic	28.72090			
Prob(F-statistic)	0.000092			

The elasticity of Cd is 3.079, which mean that if the amount of Cd increases by 1%, the rate of accumulation of metals in worms' increases by approximately 3.08%. Therefore, despite the high toxicity of cadmium, the earthworm (Eisenia fetida) is able to accumulate this metal in its body tissues [9]. The elasticity of Cr is approximately -1.214, which means that if the amount of Cr increases by 1%, the rate of accumulation of metals in worms decrease by approximately 1.21%. The elasticity of Pb is approximately -1.923, which means that if the amount of Pb increases by 1%, the rate of accumulation of metals in the worms' decreases by approximately 1.92%. The elasticity of Zn is -1.759, which means that if the amount of Zn increases by 1%, the rate of accumulation of metals in worms' decreases by approximately 1.76%. In this model, bioaccumulation of Cr, Pb and Zn decreases and it is possible that the interaction of metals causes this negative effect. The elasticity of Ni is 2.0, which mean that if the amount of Ni increases by 1%, the rate of accumulation of metals in worm's increases by approximately 2%. The model shows that bioaccumulation of Ni is approximately the same as the bioaccumulation of Cd.

Effect of pH, Moisture and Time: The availability of metals is affected by soil pH [10] and the results of the present research also show it. The model shows that one percent increase in pH value causes 17% increase in the rate of accumulation of metals in worms. Considering the effect of moisture, the results show that one percent increase in moisture causes approximately 10% decrease in the rate of bioaccumulation. Therefore, the use of vermicompost in soils with high content of water is not recommended. Finally, the results show that the increase in the time of exposure causes negative effect on the rate of bioaccumulation.

CONCLUSION

The results show that the activity of earthworms increases the mobility and bioavailability of the heavy metals in the soil, that previous researches showed it [4]. Decrease in the heavy metal concentration in the final vermicompost indicates the capability of Eisenia fetida to accumulate heavy metals in its body tissues. This capacity was investigated in soils and sewage sludge too [11-12].

Soil pH was previously found to affect metal availability [10] and this was confirmed in the present study. The deleterious effect of elevated moisture levels suggests that use of vermi-composting in soils with high water content is not advised. The uptake of metals shows a linear relationship with time, which some experiments show it [5]. The estimated model for the mixture of elements also shows the same results.

According to the results, the use of vermicompost for determination of environmental contaminants is feasible. The results highlight that vermicompost acts as a potential material for cadmium and nickel adsorption in the contaminated soils. In general, however, the use of vermicompost for remediation of environmental contaminants is feasible [12-16].

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