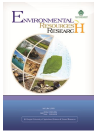

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Estimating the Effect of pH, Moisture and Heavy Metal's Interaction on Worm's Bioaccumulation Using a Linear Model

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Abstract

In the process of vermicomposting, the earthworms *Eisenia fetida* have been exposed to various metals with different concentrations to investigate the effects on worms' bioaccumulation. The study was carried out within 14 days of exposure. Five different chemical compounds in various concentration levels were added as mixture solutions to the experimental containers. Metals concentrations were measured in earthworms by atomic absorption. Ordinary least square technique was used to show metal bioaccumulation in the samples. The model showed interaction of the metals is significant. Moisture in soil had a negative effect on the rate of bioaccumulation. In contrast, pH demonstrated a large positive effect. In the experiments, worm bioaccumulation was significant for Zinc, Nickel, the most toxic metals such as Cadmium, and Chromium. Results led to a linear association between the bioaccumulation and the amount of heavy metals given the interaction as well as the rate of body burden of the worms which in turn, was affected by pH and moisture content.

Keywords: Heavy metals, *Eisenia fetida*, Bioaccumulation, Ordinary least square technique

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1. Introduction

Attempts for decreasing and eliminating pollutants from the environment, especially by heavy metals, should be taken seriously. Physical, chemical, and biological methods may be used to achieve this objective. One of the best biological methods is to hire earthworms to clean up the soil from various pollutants such as heavy metals through the process of vermicomposting. Vermicomposting is one of the best ways to dispose the wastes not only for reducing the wastes, but also for its adequacy to treat and amend the soil (Govindarajan *et al.,* 2010). Microorganisms in the digestive system of earthworms metabolize and transform organic compounds into more stable and nontoxic products. Recent experiments distinguished the capability of earthworms in accumulating heavy metals in their bodies from the materials in the soil and combining them with other compounds to reduce their toxicity (Uzoije *et al.,* 2013). As a result, it causes deduction in soil pollutant contents too.

One promising way to determine acceptable levels of pollutants in soil, lies in the application of Eco-toxicity tests, a series of internationally accepted test protocols for toxicity developed by organization for economic cooperation and development (Earthworm, Acute Toxicity Tests, OECD. 2004). Toxicity of heavy metals to various earthworms in different vermibeds has been studied by researchers (Hobbelen *et al.,* 2006; Li *et al.,* 2010). Chronic toxicity of nickel was assessed using standard test protocols for the three invertebrates *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida,* the presented data can be considered as a step toward assessment of the potential risks of nickel in terrestrial environments (Lock and Janssen, 2002).

The effect of earthworm (*Eisenia fetida*) activity on soil pH, dissolved organic carbon (DOC), microbial populations, and bioavailability of heavy metals was investigated using pot experiments. Results demonstrated that earthworm activity increases the mobility and bioavailability of heavy metals in soils (Sizmar and Hodson, 2009). Effect of mineral form of lead toxicity show the only parameter sensitive enough to distinguish the toxicities of the compounds was cocoon (egg) production. Toxicity test shows that the concentration of lead in the worms' body varies over time (Davise, 2003). Indication and uptake of the metals from polluted mine soils have been assessed as well (Ruiz *et al.,* 2009; Wang *et al.,* 2009).

Eisenia fetida was selected for the present study, because it can be easily cultivated in the laboratory. An extensive database about effects of all classes of chemicals on this species is also available (Standard Guide, 2002). The important feature of the OECD worm toxicity tests is its standard practice to add the contaminant to the soil in aqueous form. Results of the previous experiments on toxicities of solid compounds are very similar to solution compounds of chemicals (Davies *et al.,* 2003). In the present experiment, the vermibed is cow manure plus polluted soil by some heavy metal compounds. During the process of vermicomposting, earthworms accumulate metals on their body and bioaccumulation causes reduction in soil metals which is affected by pH and moisture content (Macki Aleagha, 2012; Macki Aleagha and Ebadi, 2011). The objectives of this research were to measure the rate of bioaccumulation of different concentrations of various metal compounds in the mixture on *Eisenia fetida* and the effects of moisture and pH on this rate.

2. Materials and methods

Thirteen samples of different concentrations of various metal compounds were obtained. According to OECD-Guideline for testing chemicals, testing was conducted in the laboratory at 25±1°C [10]. Soils contaminated by $ZnSO_4$, $NiSO_4$, $CdNO_3$, $K_2Cr_2O_7$, and $PbNO_3$ were analyzed through dissolving in distilled water. Four replicates of each sample and control samples were obtained. All the replicates contained 100g (dry weight) sieved cow manure and non- contaminated soil () which was then contaminated by adding various chemical compounds. The amount of pH was measured by pH meter (p25 Ecomet) . Distilled water was also added to the samples 50 (w/w). Twenty-four hours prior to the start of each test, samples were prepared and then earthworms that weighted approximately 0.2-0.4g were placed on the samples. Each replicate received 10 earthworms at the beginning of the test. Test containers were made of glass and covered by plastic, but there was a chance of oxygen exchange between the substrate and the atmosphere whilst preventing the worms from escaping. Moisture content and pH of the substrate were measured before and after the experiments. Every week, 2g of homogenized cow manure was added to the samples. After 14 days, earthworm analysis was conducted on prepared samples through spectro chemical method (standard guide, 2002).

Samples were analyzed using atomic absorption (Varian spectra AA.200). Effects of pH and moisture were estimated by a linear regression model. The model could estimate the rate of metal accumulation in worms' body over a period of time. Chi-square, student t-test, and F-statistics showed the accuracy of the coefficient in the model.

3. Results and Discussion

At the beginning of the experiments, the pH was about 7.30 and changed over the course of the experiments to 7.61. The percent of relative moisture was in the range of 43 ± 2. The mortality after 14 days was assessed by emptying the test medium into a glass plate, sorting out worms from the medium, and testing their reaction to a mechanical stimulus at the front end. Accumulation of the elements varies by the time and the interaction among them was shown using linear model. Table 1 shows the estimated linear model parameters.

Table 1. Primary amount of metals in soil samples mg/kg.

Sample	Element				
No.	Cd	Cr	Ni	Pb	Zn
1	81	8	64	457	96
2	83	9	95	525	121
3	84	9.5	97	527	123
4	96	10	120	634	179
5	110	12	186	690	192
6	111	12.5	188	692	194
7	112	13	263	921	316
8	200	20	382	1599	320
9	202	21	384	1601	322
10	218	22	418	2044	335
11	220	22.5	420	2046	337
12	223	24	430	2297	338
13	225	24.5	433	2299	340

Table 2. The accumulative amount of metals by earthworms (mg/kg) pH value and moisture content in each sample and worm.

Sample	Element					
No.	Cd	Cr	Ni	Pb	Zn	worm
1	0.0530	0.0001	0.0880	1.2500	0.8300	0.9361
2	0.0180	0.0001	0.0840	0.5900	0.6270	0.4743
3	0.0180	0.0020	0.0780	2.6900	0.5993	1.7852
4	0.0170	0.0001	0.1090	0.9500	0.9476	0.7571
5	0.0140	0.0070	0.0990	3.5300	0.5050	2.1451
6	0.0220	0.0240	0.1610	3.0600	0.6145	1.8954
7	0.0230	0.0001	0.3790	1.3200	0.5566	0.9193
8	0.0160	0.0030	0.0640	3.1000	0.4461	2.0339
9	0.0180	0.0610	0.0920	2.2600	0.3596	1.4918
10	0.0210	0.0030	0.1940	1.5700	0.3212	1.1203
11	0.0190	0.0001	0.1730	1.4800	0.1846	1.0399
12	0.0200	0.0001	0.1630	1.3900	0.2852	1.0156
13	0.0200	0.0001	0.2120	1.7900	0.3022	1.2989

Estimated linear model for the mixture of elements:

$R^2 = 0.91F = 13.23 \quad (1)$

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

L worm = 0.464 L W Cd Cr – 2.874 L W Pb + 0.073 L W Ni Zn + 12.175 L pH – 3.428 L mo – 0.758 AR(1)

L worm = the logarithm of the weighted mean of bioaccumulation results,
L W Cd Cr = the logarithm of the primary amount of Cd and Cr. (That means in this experiment Cd interacts with Cr and then affects the accumulation rate of metals in the worm),
L W Pb = the logarithm of the primary amount of Pb. L W Ni Zn = the logarithm of the primary amount of Ni and Zn. (That means in this experiment Ni interacts with Zn and then affects the accumulation rate of metals in the worm),
L pH = the logarithm of the pH value, and
L mo = the logarithm of the percent of moisture.

Table 3. Estimated linear model parameters and characteristics.

Dependent Variable: L R
Method: Least Squares

Variable	Coefficient	Std. Error	t-Statistic	Prob.
L W Cd Cr	0.464232	0.130576	3.555264	0.0120
L W Pb	-2.874841	0.686468	-4.187875	0.0058
L W Ni Zn	0.073013	0.025773	2.832891	0.0298
L pH	12.17551	2.361429	5.155992	0.0021
L mo	-3.428493	1.508794	-2.272339	0.0635
AR(1)	-0.758997	0.237356	-3.197713	0.0187
R-square				0.916861
S.E. of regression				0.175686
Mean dependent var.				0.199338
S.D. dependent var.				0.450003
F-statistic (prob. = 0.003424)				13.23372

Table 4. The amount of model results

Variables Sample No.	L R	L W Cd Cr	L W Pb	L W Ni Zn	L pH	L mo
1	-0.066076	9.138000	6.124683	18.98259	2.020222	3.772761
2	-0.745915	9.709185	6.263398	21.83944	2.020222	3.817712
3	0.579519	9.975062	6.267201	22.01435	2.032088	3.742420
4	-0.278247	10.50980	6.452049	24.83457	2.025513	3.744787
5	0.763195	11.68025	6.536692	27.47434	2.029463	3.681351
6	0.639430	11.89500	6.539586	27.58483	2.030776	3.747148
7	-0.084143	12.10271	6.825460	32.07188	2.012233	3.728100
8	0.709935	15.87234	7.377134	34.29509	2.018895	3.740048
9	0.399997	16.16114	7.378384	34.36229	2.028148	3.775057
10	0.113614	16.64370	7.622664	35.09108	2.025513	3.749504
11	0.039163	16.79314	7.623642	35.15478	2.010895	3.758872
12	0.015509	17.18428	7.739359	35.30970	2.020222	3.779634
13	0.240001	17.32433	7.740230	35.38600	2.029463	3.735286

For the estimated model of Table 2, R-square is 91% that indicates high percentage of the variations in the dependent variable (worms' bioaccumulation) are explained by independent variables.

The F-statistics in the estimated model is significant for all independent variables. The results show that Cr and Cd interact in this experiment, and their elasticity is 0.464. That means if the amount of Cr and Cd (regarding their interaction) increases by 1% in the soil, the rate of worm accumulation increases about 0.46%. It can be inferred that in the polluted soil, interaction of Cd and Cr strengthens accumulation. The elasticity of Pb is -2.876 that indicates if the amount of Pb increases by 1%, the rate of worm accumulation decreases about 3 %. The results show that Ni and Zn interact in this experiment and their elasticity is 0.073. It means if the amount of Ni and Zn (regarding their interactions) in the polluted soil increases by 1%, the rate of worm accumulation increases about 0.07%. The results show that one percent increase in pH causes 12 percent increase in the rate of worm accumulation and one percent increase in moisture causes about 3.4% decrease in the rate of bioaccumulation. Therefore, moisture in soil has a negative effect, but pH has a large positive effect on the rate of bioaccumulation. In the experiments, worm bioaccumulation was significant for Zn, Ni, and for the most toxic metals, Cd, and Cr as well.

Conclusion

According to the results, the use of vermicomposting for determination of environmental contaminants is feasible. It proves worms' capacity for metal accumulation, so we suggest vermicomposting to reduce soil metal pollution, especially near the mines and metal factories.

The results highlight the fact that vermicomposting acts as a potential method for the contaminated soils. However, it is important to know the variety of metals in contaminated soils since interaction of metals cause changes on the accumulation rate.

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