Biological Forum – An International Journal **8**(1): 565-569(2016)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Mercury and Lead Accumulation by *Eudrilus eugeniae* in Soils Amended with Vermicompost

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(Received 02 May 2016, Accepted 08 June, 2016)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The focus of the present study was to determine the accumulation capacity of heavy metals by *Eudrilus eugeniae* in soils amended with vermicompost. Garden waste, kitchen waste and cow dung were subjected to recycle through vermicomposting by using the epigeic earthworm *Eudrilus eugeniae*. Lead was mixed in soil and cow dung mixture at 0.99, 0.199, 0.298, 0.398 and 0.498 gm/kg and Mercury was mixed at concentration 0.99, 0.199, 0.299, 0.399 and 0.499 gm/kg of mercury concentrations respectively. It was concluded that despite low availability, earthworms in soils amended with vermicompost contain elevated concentrations of lead and mercury which can help in reducing the concentrations of heavy metals from soil. This is a useful indication to incorporate earthworms in agricultural ecosystems that are prone to heavy metals.

Keywords: earthworms, soil, heavy metals, agriculture

INTRODUCTION

The biodegradable material and organic waste is converted into nutrient rich vermicompost by earthworms that help in the development and maintenance of nutrient value of soil (Gajalakshmi et al. 2004). But the contamination of soil by heavy metals is one of the common problems which degrade the quality of soil and affect the growth of various plants and fauna persist naturally. Hence the overall quality of the soil can be enhanced with earthworms and will bioremediate the heavy metals content in the soil and vermicomposting can be a low-cost natural and reliable solution for removal of heavy metals as it will act as a substrate for the earthworms. However, a few species of earthworms have role in vermicomposting which can be used for remediation of heavy metals. Since, the earthworm species have short hatching time, good production of cocoons, rapid growth and maturation rate of hatchlings to adults (Domínguez and Edwards, 2004), hence suitable to use in vermicomposting process and thus for remediation of heavy metals. It is estimated that dermal exposure route is accounted for more than 82- 96% of the total uptake of heavy metals by earthworms (Saxe et al. 2001).

Due to bioaccumulation of heavy metals by earthworm introduced in agriculture ecosystem potential health problems caused due to heavy metal exposure can be reduced in human. Therefore, the present study was carried out to assess the accumulation capacity of heavy metals by earthworm *Eudrilus eugeniae* and their role in minimizing the impact of heavy metals in agriculture ecosystems.

METHODOLOGY

A. Study design

The earthworm species selected for the study was *Eudrilus eugeniae*, and the earthworms were obtained from Vermicomposting Unit, School of Studies Zoology, Jiwaji University, Gwalior, which had no history of input of either heavy metals or agrochemicals and were adults with a well-developed clitellum. They were carefully brought to the laboratory along with the moist soil and acclimatized under laboratory conditions in polyethylene beds containing soil and farmyard manure at a temperature of 28 ± 2ºC. Sufficient water was added to the dry soil to achieve a moisture content of 30% by weight. This was maintained throughout the experiment by adding deionised water. The worms were removed from the cultures 24 h prior to use, rinsed in water and kept on damp filter paper in the dark at 28 ±2ºC as a routine procedure to allow voiding of gut contents.

B. Experimental Setup

The investigation was carried out under laboratory conditions. The experiments were conducted in culture pots, each of capacity 3L. Soil was collected from MITS campus. Soil was dried, sieved through (2.36mm). For feed processing, 2-3 weeks sun-dried sieved (10mm) cow dung was soaked for 12 hours in water so as to attain its maximum water holding capacity (75%). In each experimental bin, 1kg of soil was mixed with 1kg cow dung in the ratio of 1:1. The bed was left for a period of 10 days as such.

To obtain experimental soil beds containing 0.99, 0.199, 0.298, 0.398 and 0.498 gm/kg of Lead and 0.99, 0.199, 0.299, 0.399 and 0.499 gm/kg of Mercury was mixed separately in 5 different pots along with control pot with no addition of lead and Mercury (Table I). The soil was thoroughly mixed to ensure a homogenous mixture. The moisture content was adjusted to 25% of the final weight in all experimental beds. The average height of each bed was 6.3 cm and temperature was maintained at 28 ± 2ºC throughout the study period. The experimental pots were left for 48 hours undisturbed prior to experimentation for softening of wastes or thermostabilization. Gut evacuated earthworms (25 per bin) were inoculated in each experimental bin along with the control beds. Exposure periods were tested for 28 days and analyzed.

Table 1: Concentration of heavy metals added to the soil.

S.NO.	Sample	Concentration (%)	Lead(gm/kg)	Mercury(gm/kg)
1	Control	control	0	0
2	Sample 1	10	0.99	0.99
3	Sample 2	20	0.199	0.199
4	Sample 3	30	0.298	0.299
5	Sample 4	40	0.398	0.399
6	Sample 5	50	0.498	0.499

Sample preparation for heavy metal analysis by AAS. Earthworms were handpicked from different experimental pots and were placed on moist filter paper to purge the culture bedding from their gastrointestinal tracts, and then the worms were collected and rewashed again, dried gently and kept in vials with caps. Then samples were digested. Acid digestion method was used for the determination of elements (Li *et al.*, 2009). In the first step, 5 ml nitric acid was added to the samples, and then 1 ml hydrogen peroxide was added 5 times. At the end, 2 ml hydrochloric acid was added, shaken and heated in each step, until a clear solution is obtained. The samples were filtered after cooling. Similarly, vermicompost samples collected from different experimental pots. Soil sample and vermicompost sample also digested for analysis of heavy metals by Acid digestion method.

Heavy metal analysis. The concentration of metals (lead and mercury) was analysed in acid digested samples of soil, earthworm and vermicompost at different concentration by atomic absorption spectrometer (AAS; PerkinElmer, Norwalk, USA) as described by AOAC (1999) methods.

Bioaccumulation factor. The bioaccumulation factor was used to determine whether the concentration of heavy metals in the earthworms was in equilibrium with those in soil amended with vermicompost. It is the ratio of the heavy metal concentration in tissues of earthworms (gm/kg wet weight) divided by the concentration of compounds in the soil (Hu *et al.* 2010).

RESULTS

(a) Determination of lead concentration in earthworm and vermicompost:

In control sample, the concentration of lead was negligible, while at concentration 0.99, 0.199, 0.298, 0.398 and 0.498 gm/kg of lead, there was significant accumulation of metal i.e. (0.021, 0.054, 0.066, 0.043, 0.001ppm was accumulated. In sample 5 and 6 there was fluctuation in accumulation of lead as it decreases in comparison to sample 5 due to toxic effect. Significant positive correlations are found between the earthworms and soil, lead concentrations. The concentration of lead increase in vermicompost and earthworms with respect to the increase in the concentration of heavy metals (Table 2).

(b) Determination of mercury concentration in earthworm and vermicompost:

In sample 5 and 6 there was fluctuation in accumulation of mercury as it decreases in comparison to sample 4, and in sample 6 all earthworms had died due to toxic effect of mercury.

Table 2: Concentration of lead in soil amended with vermicompost and earthworm.

S. No.	Sample	Concentration (%)	Vermicompost (ppm)	Earthworm(ppm)	r (0.05)
1	Control	control	0	0	0.813
2	Sample 1	10	0.039	0.021	
3	Sample 2	20	0.063	0.054	
4	Sample 3	30	0.072	0.066	
5	Sample 4	40	0.098	0.043	
6	Sample 5	50	0.098	0.001*	

*Not included in correlation factor

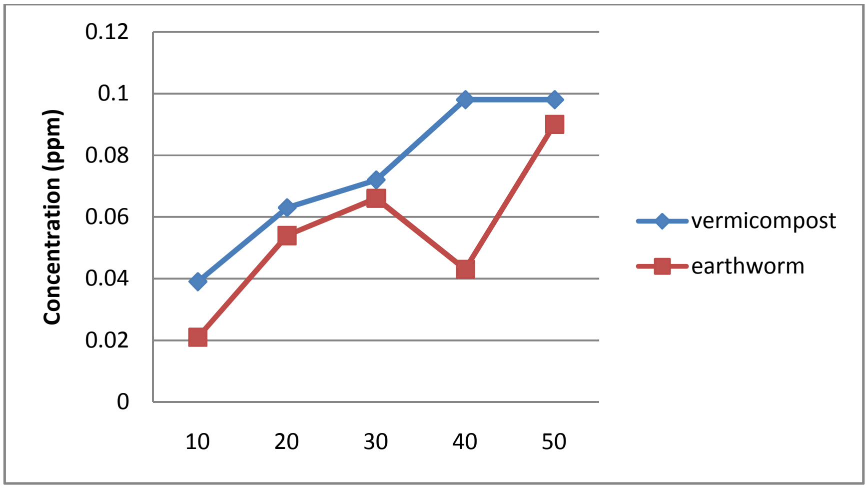

Fig. 1. Comparison of lead in soil amended with vermicompost and earthworm.

In control sample, the concentration of mercury was negligible, while at concentration 0.99, 0.199, 0.299, 0.399 and 0.499 gm/kg of Mercury, There was significant accumulation of metal i.e. (0.010, 0.015, 0.028, 0.025ppm was accumulated. Significant positive correlations are found between the earthworms and vermicompost, mercury concentrations. This indicates that the concentration of mercury increase in vermicompost as well as in earthworms (Table 3).

Table 3: Concentration of mercury in soil amended with vermicompost and earthworm.

S.No.	Concentration (%)	Vermicompost	Earthworm	r (0.05)
1	control	BDL	BDL	0.97
2	10	0.018	0.010	
3	20	0.025	0. 015	
4	30	0.038	0.028	
5	40	0.045	0.025	
6	50	0.049*	ND	

*Not included in correlation factor

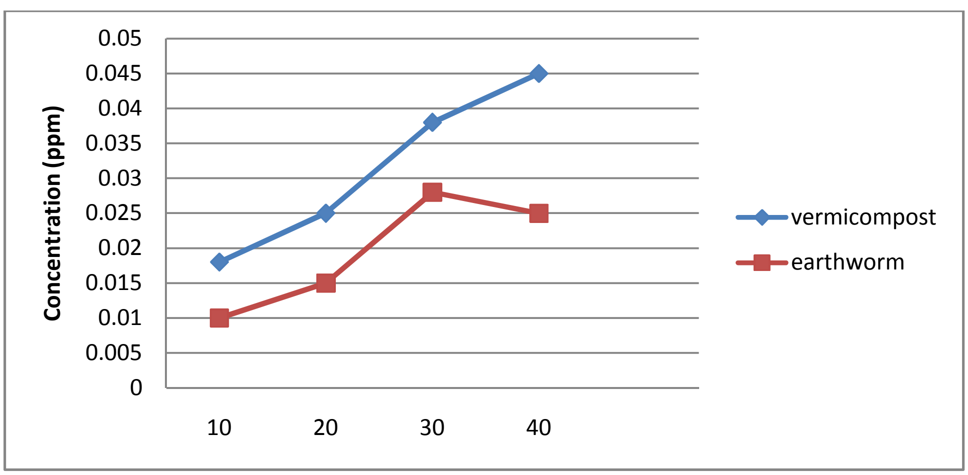

Fig. 2. Comparision of mercury in soil amended with vermicompost and earthworm.

(c) Comparison of heavy metals in soil accumulation potential by earthworm

Significant positive correlations are found between the earthworms and vermicompost lead and mercury concentrations (Table 4). The relationships were not linear (Fig. 1-3), and the accumulation patterns for lead and mercury were not similar and there was a difference in the mean metal concentrations accumulated by the earthworms in this study.

Earthworm lead concentration exceeded that of the soil, and we were unable to find the mercury concentration in earthworm at concentration of 50% due to their death. The average concentration of lead and mercury that were accumulated at different concentrations in earthworm was 0.73 and 0.611 ppm respectively (Table 4).

Table 4: Bioaccumulation factor of lead and mercury in earthworms.

S.No.	Concentration (%)	Pb (ppm)	Hg (ppm)
1	control	ND	ND
2	10	0.538	0.555
3	20	0.857	0.6
4	30	0.917	0.0736
5	40	0.438	0.555
6	50	0.010	ND
Average		0.552	0.611

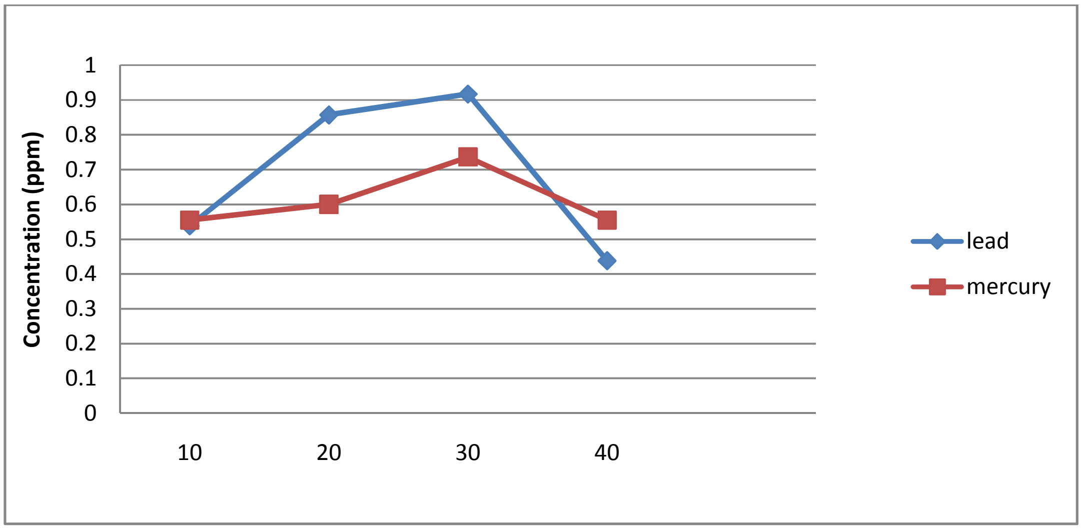

Fig. 3. Bioaccumulation factor Comparision of lead and mercury by earthworm.

DISCUSSION

The observation from the present study revealed that the concentration of the heavy metals decreased after the soil was amended with vermicompost and it was accumulated in the earthworms (Table 1-3). Lead and mercury was build up due to bio-accumulation and bio-transformation properties of earthworm as the influence of metal-contaminated soils on earthworm activity and metal bioaccumulation has been reported by different researchers (Morgan and Morgan, 1999). Earthworms can quickly occupy remediated soil (Langdon *et al.*, 2001; Spurgeon and Hopkin, 1999) and ingest soil particles and eliminate them as surface or subsurface casts. The posterior alimentary canal of earthworms is considered as a major site of metal accumulation, (Morgan and Morgan, 1989 a, b; Richards and Ireland, 1978).

The lead and mercury content were found to be reduced in soils amended with vermicompost as well in the present study (Table 2 and 3). Several studies have found that earthworms effectively bioaccumulate or biodegrade several organic and inorganic chemicals including ‘heavy metals’, ‘organochlorine pesticide’ and ‘polycyclic aromatic hydrocarbons’ (PAHs) residues (Wani, *et al.*, 2013). The possibility that vermicomposting may raise heavy metal bioavailability is of considerable relevance for the success of soil remediation, remove only part of the (presumably labile and bioavailable) heavy metals or heavy metals even remain in the soil immobilized by the addition of various chemicals (solidification/stabilization). A study carried out in the U.K. (Morgan and Morgan, 1988) earthworms (*Lumbricus rebellus* and *Dendrodrilus rubidus*) were sampled from uncontaminated and known metal-contaminated sites. Awofolu (2005) reported that the levels of lead and Cadmium in the environment have increased tremendously in the past decades as a result of human activities. Most of the machines engines run on fuel (diesel or petrol) and fuel spills from improper disposal of spent oil, coupled with pollution are responsible for the high concentration of lead and mercury in the environment.

Significant positive correlations are found between the earthworms and 'soil lead and mercury concentrations (Table 4). The relationships were not linear (Fig 2-4), and the accumulation patterns for lead and mercury were not similar and there was a difference in the mean metal concentrations accumulated by the earthworms in this study. Earthworm lead concentration exceeded that of the soil, and our study unable to find the mercury concentration in earthworm at concentration of 50% due to their death. The average concentration of lead and mercury that were accumulated at different concentrations in earthworm was 0.73 and 0.611 ppm respectively (Table 4). Soil pH coupled with a cation-exchange capacity and soil calcium had a major influence on lead accumulation and mercury accumulation may be suppressed in extremely organic soils (Morgan and Morgan, 1988). Positive correlations between metal concentrations in the earthworms and those in the soils were observed (Heikens *et al.* 2001; Lukkari *et al.* 2004). The mobility and availability of metals and metalloids in soils are generally increased by earthworms. (Sizmur and Hodson, 2009). Earthworms may also reduce the efficiency of soil remediation by mobilising recalcitrant metals (Udovic *et al.* 2007). The mechanisms for earthworm's increases metal mobility and availability are uncertain, but may involve changes in microbial populations, pH, dissolved organic carbon or metal speciation (Sizmur and Hodson, 2009).

The significant positive correlations between metal concentrations in earthworms and soil support the importance of the uptake of soluble metals from the soil. However, variation in lead and mercury concentrations in earthworm could best be explained by the absorption capacity of both the metals by the earthworm. This suggests that ingestion of heavy metals attached to soil amended with vermicompost is also an important factor determining the internal metal concentrations of earthworms. It is further concluded that the earthworms can be used in agriculture soils that are contaminated with mercury and lead to reduce the burden of heavy metals in agroecosystems.

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