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Bioaccumulation studies of heavy metal on impact towards polluted soil using earthworm *Lampito mauritii* and *Eisenia fetida*

B. Govindarajan[1*], M.Vigneeswaran[1], G.Rameshkumar[2], V.Prabakaran[3]

[1]*Venture Institute of Biotechnology, Madurai*
[2]*V.H.N.S.N College, Virudhunagar*
[3]*Govt. Arts College, Karur*

**Corresponding author, Email: vigneshwar8888@yahoo.com, Tel.: +91 9965405286*

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Abstract

Earthworms perform a number of essential functions in soil; the impacts of metals on earthworm are often investigated. Present research showed the consideration towards the range of earthworm species (*Lampito mauritii* and *Eisenia fetida),* types of soil which was highly polluted with the deposits of match industry wastes and the other site has been contaminated by wastes from automobile service station. The discharge of effluent into the open land leads to pollution of soil surrounding the service station. Henceforth, the acronyms OS and MS are found in this paper, they refer to oil polluted soil (OS) and match industry soil (MS) Control soil (CS) was collected from the surface layer of mulberry garden field and forms of metal for metal uptake and accumulation. The bioaccumulation study was carried out by employing *Lampito mauritii* and *Eisenia fetida* as test animals to three different substrates viz., CS, MS and OS for 28 days. After the completion of exposure period, the heavy metal (Cd) concentrations in the earthworm body tissues were estimated *Lampito mauritii*, grown on MS, accumulated Cd in body tissue up to a level of 0.2610 $mgkg^{-1}$, which was 6 times higher than those present at the beginning of the experiment. Similar trend was observed in another group of earthworms reared on OS. But the magnitude of Cr accumulation is only 9 times. It may be due to the high availability of this metal in the soil Similar experiment was carried out with *Eisenia fetida* on MS and OS. A significant accumulation was achieved by *Eisenia fetida* for the heavy metals Cd. When the experiment was started, the tissue concentrations of Cd in MS were 0.2484 $mgkg^{-1}$ respectively. At the end of the experimental period, the tissue concentrations of this metal were 0.3095 $mgkg^{-1}$ respectively. In the case of OS, similar trend was observed. Within 4 weeks, Cd increased drastically from 0.2484 $mgkg^{-1}$ to 0.4502 $mgkg^{-1}$.

1. Introduction

Soil is a dynamic and complex system functioning as habitat for microorganisms, plants, animals and humans. Nowadays contaminated soils have become a primordial problem since they lead to groundwater contamination and biomagnifications of chemical compounds through food webs and sometimes affect human health (Loureiro *et al.,* 2005). There is an increasing economic, political and social pressure to re-use contaminated sites. The re-use of such sites depends on, among other factors, a realistic assessment of whether pollutants at the site are present at harmful levels or not (Davies *et al.,* 2002).

Heavy metals are well known to be toxic to most organisms when present in high concentration in the environment. They affect the growth, morphology and metabolism of microorganisms in soil as they cause protein denaturation or the destruction of the integrity of cell membranes. Besides the metals of lithogenic origin, sources resulting from different human activities, such as mining, metallurgy, combustion of fossil energy sources, solid waste or sewage sludge disposal, animal effluents and fertilizer inputs for agriculture are deposited on soil. Once incorporated into the soil, they remain for every long period of time, up to several thousand years (Brookes, 1995).

Cadmium (Cd) is toxic to every system of the body, taken in via any route and plays a key role in the present day biochemical cycle of the element. Cd is a well known cumulative poison in animals. It is classified as the second most dangerous metal in our environment (Harris and Hohenemser, 1978). The main sites of Cd deposition are kidney and liver of vertebrate animals (Ferrari *et al.,* 1993). Cd has impact on soil microbiological activity, which plays a major role in soil metabolism, thus affecting soil fertility (Brookes, 1995). It is accumulated in

the soil with an average rate of 4% on arable land depending on the use of phosphate fertilizers, sewage sludge, manure and precipitation of air pollutants (Andersson, 1977). Cd is also added to soil adjacent to roads, the sources being tyres and lubricant oils.Earthworm remediation is a simple, low-cost methodology using earthworms for the accumulation of metal available in polluted sites. Earthworms are particularly suitable for the assessment of contaminant bioavailability as they are proven metal accumulators and are in full contact with the substrate they consume. The distribution of metal among the soil phases is important for the bioaccumulation by earthworms (Weltje, 1998). They are able to accumulate high tissue concentrations of trace metals from metal contaminated soils (Morgan and Morgan, 1998). They accumulate metals from soil either through direct dermal contact with chemicals in the soil or by ingestion of bulk soil or specific soil fractions. They have great potential to be used as bioassay or biomontior organisms in studies of contaminant uptake.

The objectives of the present study were to assess the efficiency of *Eisenia fetida* and *Lampito mauritii* to accumulate heavy metals in their body tissues from oil polluted soil and the soil polluted with match industry wastes and to determine the effect of the polluted soil on the growth, reproduction and mortality of *Eisenia fetida* and *Lampito mauritii*. The earthworm *Eisenia fetida* and *Lampito mauritii* were used as test animals. Total metal content of the soil was determined by Atomic Absorption Spectroscopy (AAS). The experiment was run for 28 days at 25^{o}C under dark. Growth and mortality of earthworm were measured on weekly basis. After 28 days of exposure, concentrations of total metal (Cd) available in earthworm body tissue were determined by Variant Spectra 220 (Australia) at Central Electrochemical Research Institute (CECRI) Karaikudi.

2. Materials and Methods

Test materials

Test animal

Two earthworm species namely, *Eisenia fetida* and *Lampito mauritii* were used as test animal in the present study. *Lampito mauritii* was purchased from a vermiculture farm in Madurai, Tamilnadu. *Eisenia fetida* was obtained from an earthworm bank (pit) in the Department of Zoology, VHNSN College, Virudhunagar, Tamilnadu. The earthworms were maintained in control soil which was placed in a dark room at 25 $\pm$ 2^{o}C and 80% humidity for an acclimatization period of 2 weeks.

Sampling stations

The soil samples used in the present study were collected from two sites in January 2007. Both sites are located in Allampatti, Virudhunagar, Tamilnadu. One of these two sites is highly polluted with the deposits of match industry wastes and the other site has been contaminated by wastes from automobile service station. The discharge of effluent into the open land leads to pollution of soil surrounding the service station. Henceforth, wherever the acronyms OS and MS are found in this paper, they refer to oil polluted soil and match industry soil. Soil samples were collected from the top 15 cm layer of the sampling stations. Prior to collection, plant material and litter were carefully removed from the soil. About 2 kg of soil samples were taken in individual polythene bags from both sites. Control soil was collected from the surface layer of mulberry garden field located near the Department of Zoology, VHNSN College, Virudhunagar, Tamilnadu. This soil was used for the dilution also.

Preparation of soil

Prior to analysis, the soil was air dried and passed through 2 mm sieve. It was stored in plastic bags until use. 160 g dry weight of the sieved soil was taken into each container (one liter capacity). In order to reduce the heavy metal concentration, 80 g control soil was mixed with 80 g polluted soil sample. The soil was wetted with deionized water. 40 g cow dung manure was ground, sieved and added to the experimental soil. It served as organic substrate for the growing worms.

Preparation of container

Glass containers of one litre capacity were used for the investigation. They were rinsed with distilled water. It was followed by a wash with 70% alcohol. Then they were washed with 10% HCl and distilled water. The containers were allowed to dry in air for 24 hrs.

Experimental design

To assess the bioaccumulation of heavy metals in earthworm body tissues, *Eisenia fetida* and *Lampito mauritii* were used in the experiment. Sixty clitellate *Eisenia fetida* were selected from the acclimatized earthworm stock. The worms were rinsed in distilled water to remove adhering soil particles and placed on paper towels until any excess water had drained off. The animals were divided into six groups each containing ten specimens. Each group was kept on moist paper in a petri dish at room temperature for 24 hrs to permit complete egestion of gut contents. After depuration, the worms were washed with distilled water and excess water was blotted with paper towel. The fresh weight of ten worms was determined similarly the moisture content of the soil was maintained at constant by the addition of water once in a week. Same

procedure was followed for another earthworm species *Lampito mauritii*. A replicate was also maintained for both earthworms in three different soils (ie. control soil, oil polluted soil and match industry soil).

Collection of data

Measurement of growth

Growth of earthworm was measured on weekly basis. The surviving worms were picked out and rinsed with deionized water to remove any remaining soil from their exterior before weighing. They were counted and weighed individually. The mean weight of earthworms were calculated in all replicates and recorded.

Measurement of mortality

Mortality was evaluated on day 7, 14, 21 and 28 of the experiment in all the triplicates. To check worm mortality, the test container containing the earthworms and soil were emptied onto a clean tray and the earthworms were separated from the soil. And were judged to be dead when no movement was observed after gentle stimulation with a blunt probe.

Collection of cocoon

To analyze the impact of test soil on the reproductive capacity of earthworms, cocoon production was assessed by wet sieving of the soil. Seven, 14, 21 and 28 days after addition to the soil, all the surviving earthworms were removed from the soil in the replicates. Soil was searched manually for cocoons, hatched cocoons and hatchlings.

Heavy metal analysis in soil and earthworm body tissue

All glassware were washed in 5% Nitric acid (HNO_3) and then rinsed several times with distilled water. All the three soil samples total metal concentrations of soil were obtained by acid digestion of 10 g dry weight (5 g garden soil and 5 g polluted soil) soil using 20 ml concentrated HNO_3 and 10 ml HCl. Soil sample were weighed and 20 ml HNO_3 added. The mixture in a beaker was covered with a watch glass and refluxed for 45 min. The watch glass was then removed and 10 ml HCl was added. The digested soil was them heated. During digestion, care was taken to ensure that the samples did not dry out. They were allowed to cooling before adding distilled water. 120 ml distilled water was added to makeup and filtered through Whatman filter. Then the sample was submitted for heavy metal analysis using Atomic Absorption Spectrophotometer (Varient Spectra 220) at Central Electrochemical Research Institute (CECRI), Karaikudi.

After the completion of exposure period, the earthworms were removed from the container. The animals were washed with deionised water and blotted with paper towels and weighed. The specimens were placed in petri dishes containing moist Whatman No.1 filter paper, to clear their gut content for 24 hrs. The filter paper was changed every 12 hrs to prevent coprophagy. After earthworm gut clearance, worms were rinsed with deionized water to remove any remaining soil from their exterior. Following depuration, the earthworms were kept in a hot air oven at 110^{o}C over night and the dry weight was determined. The dried earthworms were cut into small pieces and taken into the digestion tubes. They were digested using 20 ml HNO_3 and 10 ml HCl. The digests were placed on a hot plate and heated for 4 hrs at 90 to 95^{o}C. During digestion, care was taken to ensure that the sample did not dry out. After digestion, the samples were poured into 150 ml flasks through Whatman filter paper. The solution was made up to a volume of 120 ml with ultra pure mineral water. The samples were analyzed for total heavy metal (Cd) (Varient Spectra 220, Australia) at CECRI, Karaikudi.

3. Results and Discussion

Earthworms constitute a major component in soil functioning, and they play an important role in the transformation of chemical elements. Contamination of soil by heavy metals can change the functioning of soil ecosystems by disturbing the activities of soil fauna. It can lead to contamination of the terrestrial food chain. The concentrations of heavy metals in soils are widely used as a criterian for soil pollution. However, this does not provide precise information on the ability of the elements to be absorbed by plants. The influence of heavy metals in soil on earthworms and their bioaccumulation has been the subject of many studies for a long time. Several studies claimed that earthworms could serve as biological indicators of contamination because of the consistent relationship between concentrations of certain contaminants in earthworm and soil. The aims of the present investigation were to study the accumulation of some heavy metal (Cd) in earthworm body tissue; to compare the characteristics of bioaccumulation of heavy metals by earthworms and also to study the growth and reproductive success of two earthworm species *Eisenia fetida* and *Lampito mauritii*.

The bioaccumulation study was carried out by employing *Lampito mauritii* and *Eisenia fetida* as test animals. They were exposed to three different substrates viz., CS, MS and OS for 28 days. After the completion of exposure period, the heavy metal

(Cd) concentrations in the earthworm body tissues were estimated and the results are shown in Table 1 and 3. It is evident that earthworms accumulate heavy metals in body tissues from the soil. *Lampito mauritii*, grown on MS, accumulated Cd in body tissue up to a level of 0.2610 $mgkg^{-1}$, which was 6 times higher than those present at the beginning of the experiment. Similar trend was observed in another group of earthworms reared on OS. But the magnitude of Cr accumulation is only 9 times. It may be due to the high availability of this metal in the soil (Table 2).

As the availability of heavy metal was low in control soil, the earthworms could not accumulate much on their body tissues. The bioconcentration factors (BCF) for the exposure of *Lampito mauritii* of various substrates are shown in Table 2. It is evident from Table 1 that the Cd accumulation is very low by *Lampito mauritii* in both substrates.

Similar experiment was carried out with *Eisenia fetida* on MS and OS and the results are shown in Table 3. A significant accumulation was achieved by *Eisenia fetida* for the heavy metals Cd. When the experiment was started, the tissue concentrations of Cd in MS were 0.2484 $mgkg^{-1}$ respectively. At the end of the experimental period, the tissue concentrations of this metal were 0.3095 $mgkg^{-1}$ respectively. In the case of OS, similar trend was observed. Within 4 weeks, Cd increased drastically from 0.2484 $mgkg^{-1}$ to 0.4502 $mgkg^{-1}$. The control organisms did not show significant level of accumulation for all the metal tested.

The present study clearly showed that the heavy metals present in the contaminated soil were bioaccumulated in the tissues of earthworms. Similar observations were made by Kennette *et al.,* (2002). When they used *Lumbricus terrestris* for bioaccumulation study, the earthworms bioacuumulated Cu, Pb and Zn in their tissues. They reported that the earthworm tissue metal concentrations reflect the level of soil contamination. The total metal contents in the soils are positively and significantly related to the concentrations of metals in the earthworms. Similar trends for the ratios of the total metal contents in soils to earthworm contents had been observed by Morgan and Morgan (1993 and 1999). The concentrations of Cu and Pb in the earthworm tissues were of the same order than those reported from the literature for other contaminated soils by Kennette *et al.,* (2002). However, Cd concentrations of earthworms grown on OS and MS of this study are substantially higher than those recorded in other studies (Table 1 and Table 3). Considering these results, this study suggests that *Lampito mauritii* and *Eisenia fetida* can be used for the bioremediation of soils polluted with match industry wastes as well as petroleum oil products.

As the second phase of the present study, the growth pattern of two earthworm species were evaluated upon exposure to different polluted soils. The mean weight of live earthworms was calculated periodically and is shown in Table 5 and 7. There was a steady state of growth observed in body tissue of *Lampito mauritii* exposed to control soil (Table 5). At the end of the experiment, 46.55% increase in weight was noticed (Table 6). As evident from Table 5 and 6, the weight of *Lampito mauritii* grown on MS and OS showed a negative trend. As the duration of study period increased, there was a significant reduction in the biomass. This investigation revealed that *Lampito mauritii* could grow relatively well in OS than MS (Figure 1). A perusal of Table 6 indicates that the weight loss in MS is 58.69% and 32.89% in OS.

Similar trend was observed with another earthworm species *Eisenia fetida* and the results are presented in Table 7 and 8 and Figure 2. The earthworms reared on control soil showed 71.87% increase in body weight in 28 days. As evident from Table 8, MS inhibited the growth of *Eisenia fetida* significantly than OS. There are several reports which support the findings of the present study. Matuseviciute *at al.,* (2005) reported that heavy metal concentration in the substrate significantly reduced the growth of *Eisenia fetida*. They showed that Cd concentrations in earthworms had influenced their body weight. Shahmansowri *et al.,* (2005) showed that bioaccumulation of Cr, Cd, Pb, Cu and Zn by Iranian and Australian earthworms (*Eisenia fetida*) had an influence on the growth pattern of these worms. From these results, it can be suggested that the growth of *Lampito mauitii* and *Eisenia fetida* was affected by MS and OS.

The next phase of this study was to examine the reproductive success of the earthworms on different soil. As the experimental period was only 28 days, no cocoons were appeared in all the containers. It is obvious that there could be no juveniles observed during the study period. Probably, if the experimental period were extended, it would have been possible for cocoon production. This result conforms with an earlier work (Matusevicicute, 2005) in which the first cocoons were appeared after 30 days from the beginning of experiment. Moreover, the juveniles started to hatch from cocoons after 16 weeks. It was also reported that reproduction efficiency earthworm is related to the substrate nutritive value.

Table - 1 Concentration of Cd ($mgkg^{-1}$) in *Lampito mauritii* body tissue at the beginning and end of the investigation 1. Concentration of metal at the beginning of investigation 2. Concentration of metal at the end of investigation

Metal	CS		MS		OS	
	1	2	1	2	1	2
Ni	0.0887	0.0927	0.0887	0.1912	0.0887	0.2994
Cd	0.2046	0.2162	0.2046	0.2610	0.2046	0.2152
Cr	0.0553	0.0593	0.0553	1.1370	0.0553	0.5484
Pb	0.5435	0.5567	0.5435	1.5760	0.5435	0.5804

Table – 2: Concentration of Cd ($mgkg^{-1}$) in *Eisenia fetida* body tissue at the beginning and end of the investigation 1.Concentration of metal at the beginning of investigation 2.Concentration of metal at the end of investigation

Metal	CS		MS		OS	
	1	2	1	2	1	2
Ni	0.1036	0.1102	0.1036	0.1882	0.1036	0.3626
Cd	0.2484	0.2571	0.2484	0.3095	0.2484	0.4502
Cr	0.0216	0.0220	0.0216	0.0660	0.0216	0.1329
Pb	0.5848	0.5918	0.5848	0.8684	0.5848	0.6328

Table – 3. Growth pattern of *Lampito mauritii* after 28 days exposure to various substrates

Substrate	Wet weight of worms (g)		Weight loss in 28 days (%)
	Initial	Final	
CS	0.7926 ± 0.07	1.1616 ± 0.15	- 46.55
MS	0.8421 ± 0.14	0.3478 ± 0.02	58.69
OS	0.3748 ± 0.14	0.2515 ± 0.11	32.89

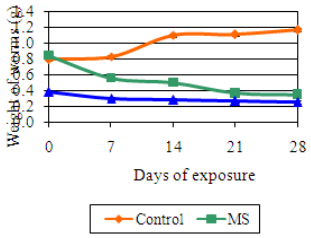

Table – 4. Growth pattern of *Eisenia fetida* after 28 days exposure to various substrates Each value is the mean ± SD of 30 individual observations

Substrate	Weight of worms (g)				
	0	7	14	21	28
CS	0.1952 ± 0.01	0.2314 ± 0.01	0.2676 ± 0.03	0.3144 ± 0.04	0.3355 ± 0.02
MS	0.2228 ± 0.03	0.2000 ± 0.02	0.1813 ± 0.02	0.1484 ± 0.02	0.1329 ± 0.02
OS	0.2126 ± 0.01	0.2103 ± 0.01	0.1945 ± 0.02	0.1848 ± 0.02	0.1672 ± 0.01

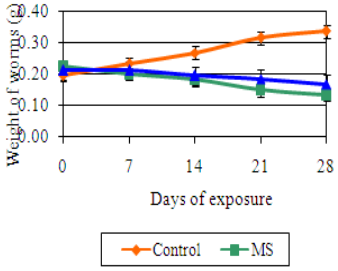

Table – 5 Weight changes of *Lampito mauritii* after 28 days exposure to various substrates Weight changes of *Eisenia fetida* after 28 days exposure to various substrates

Substrate	Weight of worms (g)				
	0	7	14	21	28
CS	0.7926 ± 0.07	0.8270 ± 0.07	1.0977 ± 0.08	1.1014 ± 0.09	1.1616 ± 0. 15
MS	0.8421 ± 0.14	0.5574 ± 0.02	0.5000 ± 0.02	0.3681 ± 0.01	0.3478 ± 0.02
OS	0.3748 ± 0.14	0.2952 ± 0.11	0.2825 ± 0.11	0.2614 ± 0.08	0.2515 ± 0.11

Substrate	Wet weight of worms (g)		Weight loss in 28 days (%)
	Initial	Final	
CS	0.1952 ± 0.01	0.3355 ± 0.02	71.87
MS	0.2228 ± 0.03	0.1329 ± 0.02	- 40.35
OS	0.2126 ± 0.01	0.1672 ± 0.01	- 21.35

Plate: 1. *Eisenia fetida*

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