Comparative Bio-accumulation Potential of Chromium and Cadmium and Their Toxic Effects on Morphology of Earthworm's Species, Pheretima posthuma

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Abstract
İn this study research was conducted to investigate the accumulative ability and devastating effect of Chromium sulfate ($CrSO_4$) and Cadmium chloride ($CdCl_2$) on the growth of earthworm (EW), *Pheretima posthuma*. An experimental trial conducted in the Labortory of the Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad. After acclimatization, earthworms reared in jars were divided into five groups with three replicates, Group A controlled, Group B was given 200mg/kg $CrSO_4$, Group C was given 300 mg/kg $CrSO_4$, Group D was given 200mg/kg $CdCl_2$, Group E was given for 300mg/kg $CdCl_2$ treated-soil, respectively. The flame spectrophotometer was used for the quantification of absorption of heavy metals. After 30 days post-exposure, the highest Cr accumulation (8.88±0.07 mg/kg) was detected in group C while highest accumulation of Cd (7.81±0.09 mg/ kg) was detected in group E. Furthermore, it was noted that these significant accumulations resulted in weight and length reduction. The highest weight reduction (-1.23±0.02g) was observed in group C while the lowest weight reduction (-1.15±0.01g) was observed in group E. Similarly, the highest length reduction (-1.82±0.03cm) was observed in group C while lower length reduction (-1.745±0.24cm) was noted in group E. It was evident that Cr has a more deleterious effect on earthworm growth than Cd. The control showed weight gain (0.26±0.05g) and length gain (1.98±0.27cm). The result of the study were statistically significant ($P<0.05$). Taken together, it was concluded that earthworm has great potential for bio-accumulation of available heavy metals (Cd and Cr) in the soil and Cr has a higher potential for bio-accumulation than Cd. Although earthworm has a great tendency to survive in a heavy metal environment, their body mass is reduced due to heavy metal toxicity. This study aims to understand the storage of heavy metals in earthworms and to investigate their bioaccumulation ability. This study aims to how heavy metals are stored in earthworms and investigate their capacity for bioaccumulation.

Keywords: *Pheretima posthuma,* Bioaccumulation, Spectrophotometer, Chromium, Cadmium

INTRODUCTION

In the terrestrial ecosystem, the heavy metals contaminated the soil due to atmospheric deposition and industrial activities (Xu *et al.,* 2017; Wei and Yang, 2019). Earthworms are essential bio indicators for the assessment of risk and have been employed in ecotoxicological research to evaluate the heavy metal potential for bioaccumulation and transmission of food chain (Peres *et al*., 2011; Suthar *et al*., 2008; Vijver *et al*., 2005; Brown *et al*., 2004) Earthworms are the vital organisms that increase the soils productivity by nutrients turnover and breaking down of organic matters into smaller units.

They increase the essential elements in the soil, which improve the plant growth (Wei and Yang, 2010). Earthworms are considered as the integral component of the ecosystems as they control the development of some micro-organisms and soil-dwelling parasites (geo-parasites). They are the most important organisms that inhabit the soil and are called "engineers of the ecosystems" as they increase soil productivity, by increasing organic matter availability through their mobility patterns, aeration, and forming pores and helps in the regulation of nutrients recycling (Demuynck *et al*., 2014; Blouin *et al.,* 2013).

Earthworms are good bio-indicators for soil contamination as they accumulate metals in their various body tissues like clitellum and gut, thus resulting in decreased environmental (soil) pollution (Nahmani *et al.,* 2007). Obviously, through these activities, earthworms are good model organisms for the bioaccumulation of heavy metals in contaminated soil (environment). Apparently, earthworm have accumulation of metals means they can accumulate these metals in crystal forms in their body tissues and the uptake of these metals is greater than their excretion (Stadnicka *et al.,* 2012). They

have characteristics to isolate these metals from the soil in their stomach instead of elimination (Pattnaik and Reddy, 2011).
Due to intense industrial development and anthropogenic activities of humans, the reasons for increased amount of heavy metals concentrations in environment are justified (Maity and Padhy, 2007). Mature earthworms are used for many Eco toxicological studies because they can accumulate large quantity of metal in their body tissues therefore, they are considered for the estimation of metal polluted environment (Li *et al.,* 2010). These metals act as essential nutrients, co factors for various organisms and plants but when they are present in (Affar *et al.,* 1998), excess amount, they proved to be harmful for those organisms and to the sustainability of ecosystem (Sari and Sary, 2014).

Many Heavy metals including, Cu, Cd, Cr, Pb and Zn readily taken up and bioaccumulate by earthworms due to their internally and externally intimate contact with soil (Nannoni *et al.,* 2014), with different tolerance capacities of contamination by different species of earthworm (Nirola *et al*., 2016; Sivakumar, 2015). Changes in the metal bio-accumulation appear to be linked to differences in metal bioavailability (Li et al., 2010; Hobbelen *et al.*, 2006), which is influenced by the environmental parameters such as redox and pH, as well as soil organic matter with metal complexation (Nannoni *et al.,* 2014; Liu *et al.,* 2017).
Metals are accumulated by earthworm species in one of two ways: through by ingestion of organic debris or by absorption by skin contact and adsorption through get tissues Hobbelem *et al*., 2016; Morgan *et al*., 2004; Vijver *et al*., 2003). Pollution due to the presence of metals in the adversely affects the productivity of crops (Uzoma *et al.,* 2013). As a result of the hesvy metals accumulated in soil, soil pores become blocked and due to this, water cannot penetrate this soil efficiently. In this way, heavy metals decrease soil productivity (Sharma *et al.,* 2005).

Intriguingly, the earthworm's body accumulate the toxicants does not only cause damage to their growth and reproduction but it also impacts some serious damage to other species of organisms like terrestrial biota (Vasseur and Bonnard, 2014; Suthar and Singh, 2009; Vasseur and Bonnard, 2014), So, EW's known as good bio-indicators for the status of the environment. Earthworm species (*Pheretima posthuma*) are widely used for metals metalation because they are present in abundance, easy to collect and handand tissues are easily separated. These organisms are easily (handled) experimentally either in the laboratory or field conditions (Affar *et al*., 1998).

Deeper understanding of the potential for food chain transmission via earthworms and other soil animals has become a primary goal in addition to concerns about heavy metal uptake by crop plants (Zhao *et al.,* 2015). Several studies have recently examined the potential risks of heavy metals and metalloids to ensure food safety, as well as the evaluation of methods for the remediation of contaminated soils (Schreck *et al*., 2012; Soek-Podwika *et al*., 2016). (Williams, 2009; Austruy *et al.*, 2013; Liu *et al*., 2013). However, relatively these studies have been observed to examine comparative bioaccumulation ability of heavy metals by the earthworm.
The major objective of this research was to evaluate the Cadmium chloride and Chromium sulphate on the physical (morphological growth) appearances, like body weight and length of earthworms; as well as the bio-accumulative ability of these earthworms for heavy metal's po heavy metal's pollution in the environment (soil).

MATERIALS AND METHODS

Collection of Experiment Animals: Seventy-five (75) adult EW (*Pheretima posthuma*) specimens were collected 3km away from-Abdullah-Pur Waterway Street Ahmad arcade in Faisalabad, Pakistan. Earthworms collected with towel were selected with well-developed clitellum, which is an indicator of their age. Waste materials like small stones, wooden material, and leaf litter were removed from the soil sample. Three-kilogram (kg) clay soil from the soil sample, was added to each of the 15 plastic containers with a carrying capacity of five liters. Earthworms were transferred into these containers and then intentionally transported to Research Laboratory alongside the wet soil from where they were collected. Then these earthworms were kept in the soil sample for their acclimatization in the dark room for 1 to 2 days. After acclimatization *Pheretima posthuma* specimens were placed in polythene containers that contain the mixtures of animal manure (cow dung) and soil. This was maintained at 25℃.

Experimental Design: The subjects (earthworms) were divided into five experimental groups with three replicates depend on the level of the heavy metal per kilogram of the soil. Group A was control setup with nothing added to the soil. In group B 200mg/kg B 200mg/kg and in group C 300mg/kg of Chromium sulphate was added to the soil. Similarly, in Group D and E, 200mg/kg and 300mg/kg Cadmium chloride were mixed respectively in distilled water. To obtain uniform (homogenous) mixture, these metals were blended with mortar and pestle in soil sample. Five subjects were transferred to each group after washing the EW's with distilled water to remove soil debris and measuring their weight and length. After 30 days post-exposure the length and weight of each subject was measured.

Heavy Metal Analysis: Heavy metal contents in the bodies of earthworms were investigated following the method is described by (Govindarajan et al., 2010), with little modification as mentioned. Subjects were placed on moist paper for 24 hours after washing with distilled water to detach the dirt particles from their bodies. To estimate the dry weight of subjects, they were kept in the oven at 110^0 C overnight. Dried subjects were cut into smaller pieces for further processing

in the digestion tubes. Dried earthworms' pieces were added in nitric acid and hydrochloric acid used in ratio of 2:1 in thirty-milliliter tube that was kept on the hot plate for four hours.
This mixture was continuously stirred and refilled with distilled water to original volume to avoid sample desiccation. A clear mixture at the end shows the completion of digestion process. After digestion, sample in tube was filtered using Whatman filter paper into the container with 50ml capacity. Distilled water was also added to raise the total volume of the mixture to 120 ml. To quantify the Cadmium and Chromium levels in the subjects, a flame absorption spectrophotometer was used.

Statistical Analysis: Data were collected in a Microsoft Excel sheet and were analyzed with Statistical Package for Social Science (SPSS) version 21 (IBM Armonk, New York, USA). Analysis of Variance (ANOVA) was used to check the difference in the body length and weight of earthworms in the control setup and across the treatment groups before and after the treatment.

RESULTS

Heavy metal accumulation in earthworms was recorded in all treatment groups after 30 days post-exposure. Results showed dosage-dependent heavy metal accumulation in the earthworm's body. Initial analysis of the Control Group revealed average Chromium (1.31±0.05 mg/kg) and Cadmium (1.19±0.05 mg/kg) levels, respectively. After 30 days of trial, the highest level (10.12±0.01mg/kg) of heavy metal (Cr) was observed in group C while in group E, the highest heavy metal (Cd) level was (9.13±0.06 mg/kg). In addition, the highest net accumulation was observed in group C (8.88±0.07 mg/kg), which was significantly higher ($P<0.05$) than all other groups. Moreover, the lowest net accumulation was recorded in group A (control group) (Table 1).

Table 1. Concentration levels (mg/kg) of different heavy metals accumulated in each of *Pheretima* species after an exposure period of 30 days.

Parameters	**A (Cr Control)**	**A (Cd Control)**	**B (Cr 200mg/kg)**	**C (Cr 300mg/kg)**	**D (Cd 200mg/kg)**	**E (Cd 300mg/kg)**
Initial Level	1.31±0.05 ^{a}	1.19±0.05 ^{a}	1.24±0.03 ^{a}	1.24±0.06 ^{a}	1.21±0.03 ^{a}	1.32±0.05 ^{a}
Final Level	1.73±0.15 ^{a}	1.63±0.15 ^{a}	8.08±0.04 ^{b}	10.12±0.01 ^{c}	7.87±0.07 ^{b}	9.13±0.06 ^{d}
Net Accumulation	0.42±0.11 ^{a}	0.43±0.1 ^{a}	6.84±0.07 ^{b}	8.88±0.07 ^{c}	6.66±0.09 ^{b}	7.81±0.09 ^{d}

Superscripts indicate significance level at 0.05 p-value.

Same superscripts mean non-significant and different superscripts mean significant.

Growth parameters were recorded after the end of trial. At the start of the experiment, the average weight of EW was noted (8.56±0.02g). The control group (8.83±0.03g) showed weight gain while all other groups showed a decrease in weight due to heavy metal accumulation. The highest weight gain (0.26±0.05g) was observed in group A (Control) which was significantly ($P<0.05$) higher than all other treatment groups. Reduction in weight was recorded in all groups: that is; B (-0.68±0.06g), C (-1.23±0.02g), D (-0.72±0.11g) and E (-1.15±0.01g). The highest reduction in weight was observed in group C which was also significant (P0.05) (Table 2).

Table 2. Weight (g) changes in each of *Pheretima* species after 30 days post-exposure period

Parameters	**A (Control)**	**B (Cr 200mg/kg)**	**C (Cr 300mg/kg)**	**D (Cd 200mg/kg)**	**E (Cd 300mg/kg)**
Initial Weight (g)	8.57±0.02 ^{a}	8.53±0.04 ^{a}	8.56±0.03 ^{a}	8.59±0.02 ^{a}	8.58±0.04 ^{a}
Final Weight (g)	8.83±0.03 ^{c}	7.84±0.02 ^{b}	7.32±0.05 ^{a}	7.86±0.09 ^{b}	7.42±0.02 ^{a}
Weight Change (g)	0.26±0.05 ^{a}	-0.68±0.06 ^{b}	-1.23±0.02 ^{c}	-0.72±0.11 ^{b}	-1.15±0.01 ^{c}

Superscripts indicate significance level at 0.05 p-value.
Same superscripts mean non-significant and different superscripts mean significant.

At the onset of experiment average body length was measured as 12.45±0.17cm, interestingly; gain in length was observed in group A (control) while all other group showed reduction in length at the end of experiment. Group A showed significantly higher (1.98±0.27) length gain. In contrast, reduction in length was observed in group B (-1.09±0.02cm), group C (-1.82±0.03cm), group D (-1.15±0.08cm) and group E (-1.745±0.24cm), respectively. Group C showed the highest reduction in length which was statistically significant ($P<0.05$) with control group. Similarly, higher reduction in length was observed in group E as compared to group A. Although, there was insignificant differences between group C and E (Table 3). This result showed the EW has very high level of tolerance for heavy metals with visible morphological changes which enables us to use EW for heavy metals accumulation bio-indicator, as well as a great tool to clear (bio-remediate) such metals. Body colour was observed to have changed from light greyish to brown after the accumulation of Chromium sulphate and Cadmium chloride metals, respectively.

Table 3. Length (cm) of the Earthworm at start and end of the experimental trial

	A (Control)	B (Cr 200mg/kg)	C (Cr 300mg/kg)	D (Cd 200mg/kg)	E (Cd 300mg/kg)
Initial Length (cm)	12.45±0.17 [a]	12.40±0.07 [a]	12.72±0.21 [a]	12.48±0.13 [a]	12.7±0.13 [a]
Final Length(cm)	14.43±0.11 [c]	11.315±0.06 [ab]	10.9±0.18 [a]	11.33±0.05 [b]	10.95±0.11 [ab]
Length Change (cm)	1.98±0.27 [d]	-1.09±0.02 [c]	-1.82±0.03 [a]	-1.15±0.08 [bc]	-1.745±0.24 [ab]

Superscripts indicate significance level at 0.05 p-value.
Same superscripts mean non-significant and different superscripts mean significant.

DISCUSSION

Heavy metal's accumulation in earthworm species takes place by the two pathways described by (Nannoni and Protano, 2016; Wei *et al.*, 2003), which include ingestion of organic material, in which adsorption takes place through the stomach tissues, or absorption through the skin. The significance of gut adsorption and skin (dermal contact) may differ depending on the diversity, ecological traits and eating habits of different species of earthworm (Nirola *et al*., 2016; Nannoni *et al*., 2011). Basically, there are three ecological groupings into which earthworm species fall. These include epigenic earthworms, which live and dig in the top layer of soil, endogenic earthworms, which inhabit soil profiles rich in organic matter, and anecic earthworms, which move both horizontally and vertically in the soil and form burrows in the lower surface. (Morgan *et al.*, 1991; Nannoni *et al*., 2014).

Different soil fractions have different distributions of heavy metals and bioavailabilities. Therefore, it may be concluded that earthworms belonging to various ecological groups will experience heavy metal exposure in numerous ways and have various levels of metal bioaccumulation (Sivakumar et al., 2015; Shuster et al., 2004; Li et al., 2010). In this study, we only used one species of earthworms (*Pehertima posthuma*) specimens to check their accumulation capacity for both heavy metals' Chromium sulphate and Cadmium chloride at two different concentrations. While in previous study conducted in china compared the bioaccumulation rates of various heavy metals using four distinct species of earthworms (Amynthas homochaetus, Amynthas heterochaetus, Metaphire California, Amynthas pecteniferus) (Pb, Cd, Cu and Zn) (Wang *et al.*, 2018).

The level of heavy metal content in the body of the earthworm was increased depending on the level of environmental heavy metal content. The earthworm's heavy metals accumulation (Chromium) at greater concentrations in their bodies showed that EW's has the capacity to accumulate Chromium to a large extent in their bodies, thus leading to a reduction in the body weight of earthworms. Investigations of (Blouin *et al.*, 2006), showed that earthworms have the various metallics distributions (Mn, Zn, Fe, Cd, Co, Ni, Pb, Cu, Hg, Ni, and Ar) as *Pheretima hilgendorfi* species of earthworms showed reduction in their body mass (length and weight) after the experimental period; this study supported our present study (Renoux *et al.*, 2007).

In this present study, we determined that both metals were accumulated at different concentrations by the same species of EW's depending on the level of metals in the soil. The studies of (Li et al., 2010; Nahmani et al., 2007 Hobbelen et al., 2006; anno *et al.*, 2004), showed that these differences in bioaccumulation of various metals also appear to be related to the metal complexation with the soil organic metter and differences in metal bioavailability (Wang *et al.*, 2018).

In this study showed that body mass of the EW was reduced after using the heavy metals in the soil. By extension, the body length was also observed to reduce. These results suggested that they have shown concentration-dependent reactions. The results of this study were in accordance with the findings of previous study which has observed the reduction in body length and weight when EW specimens were exposed under the influence of Zin, Chromium, and Cadmium (Ma *et al.*, 2002). Similar results were observed by (Zheng and Canyang, 2009). The results showed that there was decline in body weight of EW's at higher concentration of Chromium in soil. Findings from (Nahmani *et al.*, 2007), showed that the decrease in body mass of EW's was recorded after the treatment of metals. The trends in the accumulation of metals are not similar for both metals at different concentrations.

In the present study the concentration of heavy metal accumulated in the tissue of the earthworm species was highly significant, especially for Cr. In relation to this study of (Morgan *et al.*, 2001), has shown that Chromium was accumulated in greater concentration in comparison to Cadmium, although EWs were inhabiting in the same concentration of metal-contaminated soil. These results are in accordance with this present study. Effect of Cadmium chloride exposure was less severe as compared to Chromium sulphate which showed less reduction in their weight. Similar result was obtained by (Honda *et al.*, 1984) who recorded that a lesser decline in body weight of *Pheretima hilgendorfi* was observed under lower Cadmium chloride concentration as compared to Chromium and Zinc; this study showed similarity with our present study.

The mineralization of dead earthworms will release accumulated heavy metals into the environment, and the chemical changes that occur in the earthworm's digestive tract may make various metals more susceptible to plants (Zhao *et al.*, 2015). The detoxification mechanism within the earthworms varies with the concerned metal but the accumulation of metals at moderate levels for reasonably very specific and short periods appears to have a deleterious effect o growth or biomass and no mortality can occur (Wang *et al*., 2018; Austruy *et al*., 2013). In the present study, heavy metals accumulation showed a reduction, both in body length and weight of earthworms, which are the deleterious effects of metals and no mortality occurred both in the control and treatment groups.

CONCLUSIONS

It can be concluded earthworm (*Pheretima posthuma*) has a tendency to uptake the heavy metals in the soil thus clearing (bio-remediating) the environment from heavy metals pollution. Uptake of heavy metals by EW'S related to matal's concentration in the environment. The EW'S has a great tendency to survive in a heavy metal environment, but their body mass is reduced due to heavy metal toxicity. To identify the likelihood of food chain transmission and the importance of other species for risk assessment in heavy metal polluted soils, further research into the metal storage and detoxifying mechanisms in other species is needed.

Conflict of Interest: According to the author, there is no conflict of interest.

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