

Efficacy of *Pheretima* species for accumulation of heavy metal contaminants

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Abstract- The goal of present review was towards impacts of overwhelming metals on varying qualities of earthworms in addition to accumulative capability of heavy metals like Pb (NO₃)₂ and ZnSO₄. The present review deals with *Pheretima* species that were chosen for the assurance of substantial metals bioaccumulation. Biomass of worms recorded before the begin of examination and contrasted with the last biomass following 4 weeks, a critical lessening stemmed while increment in biomass was seen in charge earthworm. A steady reduction was seen in the length of *Pheretima* types of worms in treated investigations. Increment long of night crawlers watched for control try like 12.6cm to 13cm. They were provided with zinc sulphate (300, 200mg/kg of soil), lead nitrate (300, 200mg/kg of soil). When trial duration over, flame absorption spectrophotometer was used to analyze the accumulation of heavy metals in earthworms and soil samples. It was apparent that the take-up of zinc by *Pheretima* species happened and the most noteworthy amassing found was 1.747mg/kg. Worms assume a critical part by gathering overwhelming metals from debased soil and clean condition. Maximum accumulation was observed for zinc with mean value 74mg/L and lead with 4.2mg/L in sewage water.

Index Terms- Overwhelming, Accumulation, *Pheretima* species, Spectrophotometer, Sewage water.

I. INTRODUCTION

The foremost ideas to conduct this study were to govern the effects of lead and zinc which are considered as heavy metals on physiology, morphology, demography of earthworms and measure the accumulation ability of *Pheretima* species of earthworms. Earthworms are helpful in land recovery, soil change and basic waste association (Harender and Bhardwaj, 2001). Worms assume vital part as bio-control by helping the development of ideal microscopic organisms and parasites living in soil Pfiffner (2014). The deterioration of natural matter night crawlers assume a fundamental part in soil digestion through fracture, encouraging, air circulation, scattering and turnover (Shuster *et al.*, 2000). Among few spineless creatures, worms have focal sensory system with well create cerebrum Priyadarshini (2015). They can't smash roots as they don't have teeth (Dominguez *et al.*, 2015). Their offsprings achieve development at the age of sixty to ninety days. Normal expected life span of a night crawler is about four to eight years. In their body tissues earthworms can store up the metal particles since worms are best bio pointers of take after metals among soil

cowardly animals (Nahmani *et al.*, 2007). They can isolate overwhelming deposits by leaf mold or organic fertilizer in abdominal region which they later store in their bodies somewhat discharging out (Patnaik and Reddy, 2011). Due to rapidly creating organizations in making countries in this way pollute the water and air considerable metals are supplemented with high concentrations (Maity *et al.*, 2008). Earthworms show change in their body structure in loams familiar with overpowering metals so they are bio markers of trademark framework dauntlessness (Li *et al.*, 2010). In soil the general substantial metal substance is commonly utilized as a constraint for portraying pollution of dirt (Sary and Sari, 2014). Availability of metals to living creatures is named for example the amount of pollutants that is accessible to alive portion of soil (Ehlers and Luthy, 2003). Simply living creatures are used to choose bioavailability of significant metals in the soil Harmsen (2007). Accumulation implies overwhelming metals heap active in figures by great degree formerly before releasing (Stadnika *et al.*, 2012). Soil balanced with significant metals impacts the natural conditions similar to quality (Morgan and Morgan, 1991). Generous metal storing up in soil organic frameworks meddle with the improvements and activities of animals living in them and bring about decreasing proficiency as by dirt (Sharma *et al.*, 2005). Overpowering mixtures release extra expeditiously in sour standard and rinses significant covert liquid in this way comes to fruition bona fide risks for dirt animals and vegetation (Buttona *et al.*, 2010). A suggestion identified with substantial metal pollution is a noteworthy risk for the most part for creation of farming framework (Uzoma *et al.*, 2013). Bioaccumulative examinations of night crawlers are to a great degree profitable in choosing the total method for considerable metals in animated creatures to review the mind-boggling metals in without ecological possessions (Piercea *et al.*, 2002). Dirt contaminated by intense metals bearings particular position of earth becomes similar to eminence (Spurgeon *et al.*, 2003). Vermicomposting, by means of worms, in ecosystem prepare that changes vitality rich and intricate natural substances into a balanced out fertilizer-like item (Benitez *et al.*, 2000). Earthworms have connotation with atmosphere and forage on microbiological dirt of ecosystems (Pokarzhevskii *et al.*, 1997). Heavy metals dissemination to earthworms after conduction increases significantly their bioavailability (Ma *et al.*, 2002; Wen *et al.*, 2004; Cheng and Wong, 2002). The foremost ideas to conduct this study were to: govern the effects of lead and zinc which are considered as heavy metals on physiology, morphology and demography of earthworms, measure the accumulation ability of *Pheretima*

species and also determine heavy metals in sewage water on *Pheretima* species.

II. MATERIALS AND METHOD

Earthworms are vital species in numerous biological units and pronouncedly affecting rotting dynamic supplement mineralization and essential generation. The earthworms for the investigation were gathered inside three km region from Ahmad arcade Abdullah pur waterway street Faisalabad as the dirt here was not sullied with overwhelming metals. Twenty five individual night crawlers of *Pheretima* species, with very much created clitellum were inspected from the upper layers of sodden soil with the assistance of towel and soil was also gathered from top strata of dirt ten centimeter profound. Vegetative factual and brood expelled in dirt. 3 kilogram dirt endured in 5 elastic compartments or holders with the capacity of five liters. At that point they were exchanged to research center deliberately alongside soggy soil from where they were gathered. The worms then were placed in appropriate conditions inside sample soil for adjustment in a dim space for one to two days.

With the end goal of acclimatization the *Pheretima* species were kept in research facility in polyethylene compartments that have soil and dairy animal compost at a temperature of about twenty five with little bit difference of two degree centigrade. They were weighted and measured at the end of trail period of about 30 days. Two different heavy metals like lead nitrate and zinc sulphate of 300mg and 200mg concentrations were used.

Basic design of experiment

A sum of five investigations was directed with one control and four medications with three replications. To get trial beds, soil was treated with fitting measure of substantial metals. 300 and 200 milligram per kilogram zinc sulfate also 300 and 200 milligram per kilogram lead nitrate for per kilogram of soil were independently blended in refined water. These substantial metals arrangement was included soil and blended altogether to acquire homogeneous blend. In first holder (T1) or treatment one zinc sulfate of 300 milligram per kilogram fixation was included, in second compartment (T2) or treatment two 200 milligram per kilogram centralization of zinc sulfate was included and in third holder third (T3) or treatment three 300 milligram per kilogram of lead nitrate and fourth compartment (T4) or treatment four 200 milligram per kilogram grouping of lead nitrate was included. Fifth pot was utilized as control.

With a specific end goal to check the bioaccumulation of substantial metals in the body tissues of earthworms, seven grown-up worms from acclimatized worm stock were included every holder. Before staging of earthworms into these containers or compartments firstly washed through liquid completely for the expulsion of particles stick on the bodies of earthworm, afterward placed into channel rag till profusion of liquid that is water remain soaked. Departure of gut substance can be made by putting the earthworms of *Pheretima* species in hygienic slides for one night. From point of forward, every worm was weighed and measured and after that additional to compartment, secured with top and afterward the holders were kept in lab for 30 days.

Diagnostic procedure for metal accumulation in *Pheretima* species of earthworms

To determine the substantial metals accumulation in body of *Pheretima* species of earthworms, put on wet channel paper for one day to remove instinctive material of earthworms. Afterwards every worm was rinsed by liquid that is water to expel additional matter as well as dirt substance which adhere to the body of *Pheretima* species of earthworms. Worms yielded via observance the earthworms on cutting-edge broiler on 110 degree centigrade then at last the dehydrated mass recorded. To do absorption, the desiccated worms censored to little parts for the exchange of earthworms into processing cylinders. An answer to use nitric acid as well as hydrochloric acid set up a trendy proportion of two ratio one in thirty milliliter tube that kept in warm dish for almost four hours. Arrangement of completely blended was done and care taken to blend not desiccated. When assimilation was done the specimen was emptied via Whatman channel sheet intone hundred and fifty milliliter limit bottle and the volume was raised to one hundred and twenty milliliter through the expansion of refined water. According to (Govindarajan et al., 2010) the processed specimens of night crawlers were examined through spectrophotometer for substantial metal collection.

Statistical Analysis:

Information was examined factually through ANOVA and correlation. Correlation was used to check the accumulation of substantial metals in *Pheretima* species in connection to bioavailable overwhelming metals in soil and in the assortments of worms. To check change in morphological parameters (length and weight) after treatment application, analysis of variance was practiced.

III. RESULTS

Consequences of the underlying as well as last weight demonstrated worm mass body was diminished while earthworms amassed metals overwhelmed. More huge reduction happened with 300mg fixation treatment when weight was decreased to a substantial degree. Mass of worms noted and analysis of heavy metals was done and contrasted with the last mass following week four, a huge decrease curtailed whereas rise in body mass was seen in charge *Pheretima* species (Table 3.1). A significant relationship was found when Analysis of Variance was applied between weight and treatment (Table 3.2). More huge reduction happened with 300mg fixation treatment when length was decreased to a substantial degree. Length of worms noted and analysis of heavy metals was done and contrasted with the last length following week four, a huge decrease curtailed whereas rise in body mass was seen in charge earthworm species of *Pheretima* (Table 3.3). When ANOVA was applied on the initial and final values of length then significant results were obtained (Table 3.4). Table 3.5 describes the relationship among length and weight of *Pheretima species* of earthworms on exposure to heavy metal treatments. The convergence of lead and zinc metal in treatment of 5 individuals of *Pheretima* type were examined through fire ingestion spectrophotometer. Collection of lead and zinc was seen in all repeats of worms. It was apparent that the take-up of zinc by *Pheretima* species happened and the

most elevated collection found was 1.747mg/kg. The convergences of lead (0.985mg/kg) were not like zinc metal treatment as lead aggregation examination showed less capacity of worms to amass in their bodies when contrasted with zinc (Figure 3.1). The sewage water contaminants on *Pheretima* species were also checked and water was collected from nearby industry impurities. Lead and zinc was found with 4.2mg/L and 72mg/L by Atomic Absorption Spectrophotometer (Figure 3.2).

IV. DISCUSSION

Worms assume an essential part in keeping up the strength of soil. The earthworms have capacity to change over the undesirable natural matter into helpful natural fertilizer. They give valuable outcomes in soil advancement, arrive recuperation and association of natural waste (Harender and Bhardwaj, 2001). The division of overwhelming metal present in soil modules describes the most essential elements influencing their adaptability in soil and acknowledgment by marine and freshwater shellfish (Dai et al., 2004; Lanno et al., 2004; Becquer et al., 2005 and Hobbelen et al., 2006). There are numerous substantial metals which are broadly degree in soils e.g. Zn, Pb, Cr, Cd and Cu. Overwhelming metals are deadly to numerous life forms past specific points of confinement and divulgence times can influence the living beings plenitude, dissemination and assorted qualities (Hopkin, 1989 and Lukkari et al., 2004). In spite of unsafe impacts, metals don't stop in soil however overwhelming metals announcement to the biological system may be constrained (Brusseu, 1997). The previously mentioned outcomes unequivocally bolster the investigation of (Ma et al., 2002) in which they watched lessening in weight in *Pheretima* types of night crawlers when presented to lead and zinc mine tailings. The marginally unsafe impacts might be prized signs on night crawlers because of metallic toxins as portrayed by Donker 1992; Ma 1984. Comparable decline in body weight of night crawler *Pheretima guillelmi* at high centralization of lead in soil has been recorded by (Zheng and Canyang 2009). At the point when earthworms morphological characters were broke down there was noteworthy connection between the abatement long and weight with time. In a comparative review led by (Nahmani et al., 2007) generous decrease in body mass of night crawlers recorded in worms in substantial metals stacked soil. Earthworms have ability to accumulate maximum concentration of zinc metal in their bodies and earthworms also have capability to normalize the concentration of zinc regardless of the quantity present in soil (Renoux et al., 2007).

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Tables of article:

Table 3.1: Variations in weight of earthworms (*Pheretima* species) when exposed to different treatments.

| Control | | Treatments | | | | | | | |
|--------------------|--------------------------|--------------------|--------------------------|--------------------|--------------------------|--------------------|--------------------------|--------------------|--------------------------|
| | | Lead nitrate | | | | Zinc sulphate | | | |
| | | 300mg | | 200mg | | 300mg | | 200mg | |
| Initial weight (g) | Weight after 30 days (g) | Initial weight (g) | Weight after 30 days (g) | Initial weight (g) | Weight after 30 days (g) | Initial weight (g) | Weight after 30 days (g) | Initial weight (g) | Weight after 30 days (g) |
| 7.94 | 8.35 | 8.80 | 8.53 | 7.98 | 7.73 | 7.87 | 7.62 | 7.62 | 7.56 |
| 8.12 | 8.56 | 8.98 | 8.70 | 8.00 | 7.89 | 7.71 | 7.58 | 7.51 | 7.45 |
| 7.79 | 7.85 | 8.35 | 8.24 | 7.89 | 7.64 | 8.16 | 7.98 | 7.39 | 7.32 |
| 7.88 | 7.97 | 8.31 | 8.00 | 8.01 | 7.92 | 7.79 | 7.56 | 7.49 | 7.15 |
| 7.57 | 7.66 | 8.32 | 8.14 | 7.97 | 7.76 | 7.45 | 7.23 | 7.50 | 7.26 |

Table 3.2: ANOVA table show the weight of *Pheretima* species of earthworms after treatment exposure period.

| S.OV | D.F | SS | MS | F-value |
|-----------|-----|--------|-------|---------|
| Weight | 1 | 2.231 | 2.231 | 8.647** |
| Treatment | 4 | 1.520 | 0.38 | 1.472** |
| Error | 44 | 11.362 | 0.258 | |
| Total | 49 | 13.592 | | |

Table 3.3: Variations in length of earthworms (*Pheretima* species) when exposed to different treatments.

| Control | | Treatments | | | | | | | |
|----------|---------|--------------|---------|---------|---------|---------------|---------|---------|---------|
| | | Lead nitrate | | | | Zinc sulphate | | | |
| | | 300mg | | 200mg | | 300mg | | 200mg | |
| I. L(cm) | F.L(cm) | I.L(cm) | F.L(cm) | I.L(cm) | F.L(cm) | I.L(cm) | F.L(cm) | I.L(cm) | F.L(cm) |
| 12.6 | 13 | 14..3 | 14 | 13 | 12.6 | 12.7 | 12 | 13.6 | 13 |
| 11.8 | 12 | 13.7 | 13.2 | 13.7 | 13.1 | 14 | 13.2 | 14.1 | 13.4 |
| 12.2 | 12.8 | 12.8 | 12.1 | 12.9 | 12.3 | 12.6 | 11.9 | 12.7 | 12.3 |
| 11.1 | 11.8 | 13 | 11.9 | 11.9 | 11.1 | 14.7 | 13.9 | 12.4 | 11.1 |
| 13 | 13.4 | 12.7 | 12 | 13.9 | 13.1 | 12.8 | 12.2 | 12.8 | 12.6 |

I.L= Initial Length F.L= Final Length

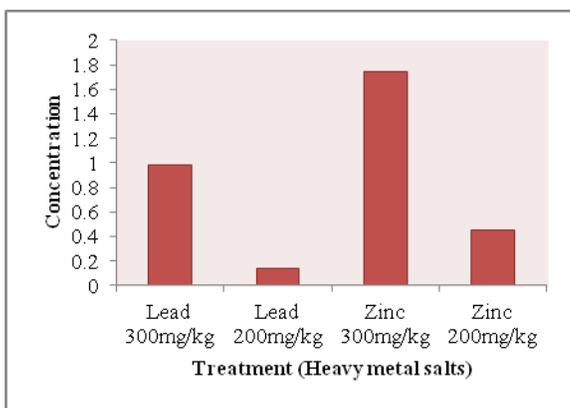
Table 3.4: ANOVA table show the length of *Pheretima* species of earthworms after treatment exposure period.

| S.OV | D.F | SS | MS | F-value |
|-----------|-----|--------|-------|----------|
| Length | 1 | 6.743 | 3.371 | 10.389** |
| Treatment | 4 | 1.323 | 1.323 | 2.038* |
| Error | 44 | 29.857 | 0.649 | |
| Total | 49 | 37.60 | | |

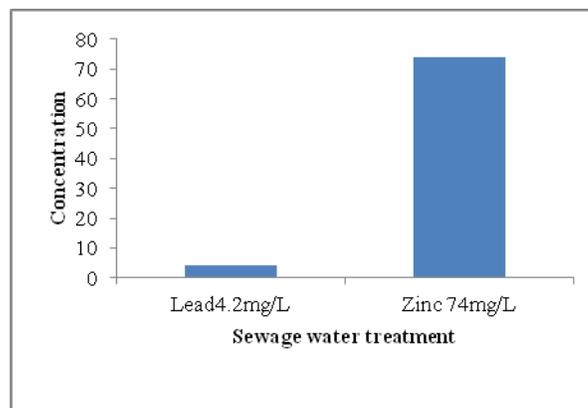
Table 3.5: Relationship among length and weight of *Pheretima species* of earthworms on exposure to heavy metal treatments.

| Treatments | Correlation | (p value) |
|---------------------|-------------|-----------|
| Control | -0.284 | 0.426 |
| Lead nitrate 300mg | 0.857* | 0.002 |
| Lead nitrate 200mg | 0.168 | 0.643 |
| Zinc sulphate 300mg | -0.011 | 0.977 |
| Zinc sulphate 200mg | 0.783* | 0.007 |
| Total | 0.155 | 0.282 |

When $p < 0.005$ then results were significant.



(Fig 3.1) Heavy metal accumulation variations in four different sewage water experiments.



(Fig 3.2) Comparison of different heavy metals from that were accumulated in earthworms.