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Bioaccumulation Pattern of Heavy Metals in *Solanumlycopersicum* Co-Inoculated with Red Worm *Eiseniafetida*

PattukumarV[1]., Anantha SelviG.S[1]., Sivakumar P[2]., Vasanthakumar P[3]., Susanna Roy[4]., AshaAnand[4]*.

[1]P.G Research & Department of Zoology, S.T. Hindu College, Nagercoil, Tamil Nadu, India,
[2]Research Department of Zoology,The Madura College (Autonomous) Madurai, Tamil Nadu, India,
[3]Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India,
[4]Department of Life sciences, CHRIST (Deemed to be University), Bangalore, Karnataka, India.

**Corresponding author's E-mail:* asha.anand@christuniversity.in

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Abstract

The presence of heavy metal in the environment has been increasing with the increasing anthropogenic activities. The following study observes the bioaccumulation activities of Solanumlycopersicum, Eiseniafetida and a combined set-up containing both. The metals used for the particular study includes lead, mercury, chromium and cadmium. It was observed that each heavy metal has a particular pattern of accumulation as well as this pattern being affected when in the combined set up containing both Solanumlycopersicum and Eiseniafetida. In the end the combined set up was more efficient in bioaccumulating heavy metals from the contaminated soil.

Keywords: *Anthropogenic, Heavy metals*

1. Introduction

Heavy metal pollution is increasing with the intensification in the industrialization process. Man's greatest challenge was to speed up industrialization previously but now they are finding ways to deal with the problems growing with the industrialization[1].The rapid introduction of pollutants into the ecosystem has overwhelm the self-cleaning capabilities of the ecosystem due to which there has been an high accumulation of the pollutants. Soil naturally has a certain amount of heavy metals in them which are essential for the living systems as co-factors for metalloproteinase and enzymes. But these metals can also cause deleterious actions by blocking essential functional groups and modifying certain conformation of biological molecules [2]. Heavy metals are very unique as they do not undergo any chemical or biological degradation hence it gets accumulated in the ecosystem. Although plants might suffer the damages from excessive contact with heavy metals, plants require these metals for the formation of micronutrients for the growth and development[3]. Remediation of such contaminated soil is expensive if done with the traditional physiological processes. Hence with the increase in the pollution in the ecosystem there is a need in the bioremediation of these contaminated areas using method such as Phyto-extraction, Phytostabilization, phytostimulization[4].

Earthworms are reported for their ability to aid in removing several kinds of inorganic as well as organic materials from the soil.[5]. The mechanisms via which the worms are capable of doing so include biodegradation, biotransformationby absorption by skin or the intestine [6]. Species such as *Eiseniafetida, Aporrectodatuberculata, Lumbricusterrestris, Lumbricusrubellus, Dendrobaenarubida, Dendrobaenaveneta, Eiseniellatetraedra, Allobophorachloritica, and Libyodrilusviolaceu* are reported as better metal sequesters [12, 11]. The major detoxification method in earthworms include induction of metallothionesis and sequestration of these within the body [7]. Heavy metal stress is neutarlised by metal immobilization within their body or by storage as brown bodies or excretion via calaferous glands [12]. Bioremediation of soil using worms include the direct application of worms onto the soil, application of the contaminated media as the feeding regime and lastly the use of vermidigested material [8].Combination studies to reduce heavy metal concentration within the soil have already been conducted with plants like *Lantana camara*andearthworm *Pontoscolexcorethrurus, Paulownia tomentosa*and*Cytisusscoparius*plants with *Eiseniafetida, Hordeumvulgare* and

Lumbricusspsignifica[18, 19, 20].Our research was focused to test the bioremediation quality of *Solanumlycopercicum* and *Eiseniafetida*on artificially contaminated soils.

2. Materials And Methods

One-week old*Solanumlycopersicum* were purchased from Manvik nursery, Bangalore. Adult Earthworm (*Eiseniafetida*) was collected from the Bhanerghatta Bio-center Bangalore. The solution mixture containing heavy metalof lead acetate, mercuric chloride, chromium trioxide and cadmium sulphate was prepared at a total concentration of 160mg/l, 320mg/l and 640mg/l and were used in artificial contamination studies.Supplementation of heavy metals was carried out daily. After two weeks of heavy metal treatment, leaves of the saplings were collected and used for acid digestion.

Acid digestion of leaves

The acid digestion was done in accordance with the method formulated by Pequerul et al [21]. The gathered leaves were dried in an oven.0.05g, with 4ml of nitric acid being used for digestion. Using a hot plate, the solution was heated to 120°C after being incubated for the entire night. After cooling the sample, 2 millilitres of 33% hydrogen peroxide were added, and the sample was left colourless. Next, distilled water was added to the solution to make it up to 25ml. Atomic absorption spectroscopy was used to assess the concentration of heavy metals. Every sample reading is a representation of the triple data.

Accumulation studies in one month old *Solanumlycopersicum.*

Four-week-old *Solanumlycopersicum* were used in accumulation studies. The plants were potted in glass jars containing 300g of soil. 20ml of the solution of concentrations 160mg/l, 320mg/l and 640mg/l of heavy metals were added to the respective jars and incubated for two weeks. All studies were carried out in triplicates. The leaves of the plant were collected and were acid digested for heavy metal analysis of lead, mercury, chromium and cadmium.

Sequestration study in *Eiseniafetida*.

The compost was prepared using 1:1 ration of dry cow dung and dry leaves. The compost was watered and mixed daily for two weeks until it was moist enough for the consumption by earthworms.300g of the compost was taken in glass jars. 20ml of the heavy metal solution of the concentration 160mg/l, 320mg/l and 640mg/l were added into the respective glass jars. After a day of incubation, soil was collected before the introduction of earthworms and was marked as day 0 value. Worms were introduced in each jar and was closed using jute rags to prevent worm escaping from the microenvironment. Worms were collected along with the soil after exposing them for two week. The soil was acid digested using nitric acid attack method where 0.5g of soil was taken in the test tube with 2.5ml of nitric acid and heated up to 105°C. The cooled solution was made up to 25ml with water and was used for AAS analysis[22].

Heavy metal accumulation in earthworm tissue was analyzed using ash method for acid digestion. The samples were digested in accordance with Kartz and Jennie's method [23]. Where 0.5g of the tissue was made into ash using a furnace at 300°C. To the collected ash, 10ml of 55% nitric acid solution was added and kept for overnight digestion. The mixture was then heated at 40-60°C for two hours and heated to 120-130°C for one hour. The mixture was cooled down and 1ml of per chloric acid was added and heated at 120-130°C for an hour. The solution was then made up to 25ml using distilled water and was analysed using AAS.

Sequestration studies in both plant and earthworm

Twenty ml of the heavy metal solutions of concentration 160mg/l, 320mg/l and 640mg/l was added into the respective jars containing 300g of soilprior to the addition of the worm or tomato plants. The soil collected prior to addition of worm and plant was termed as day 0sampling. Four week old tomato plant was transplanted into the glass jars to which earthworms were added. The jars were tightly wound using aluminum foil and incubated for two weeks. After two weeks of incubation, leaf, soil and earthworm were collected for the acid digestion and metal analysis using AAS.

Statistical analysis

One tailed T test was conducted for testing the significance from the control to the treated samples. The T test was conducted for each metal for leaf and worm samples. The formula used for one tailed T test is as follows. ANOVA was conducted for finding the significance between all the three set-ups.

3. Results and Discussion

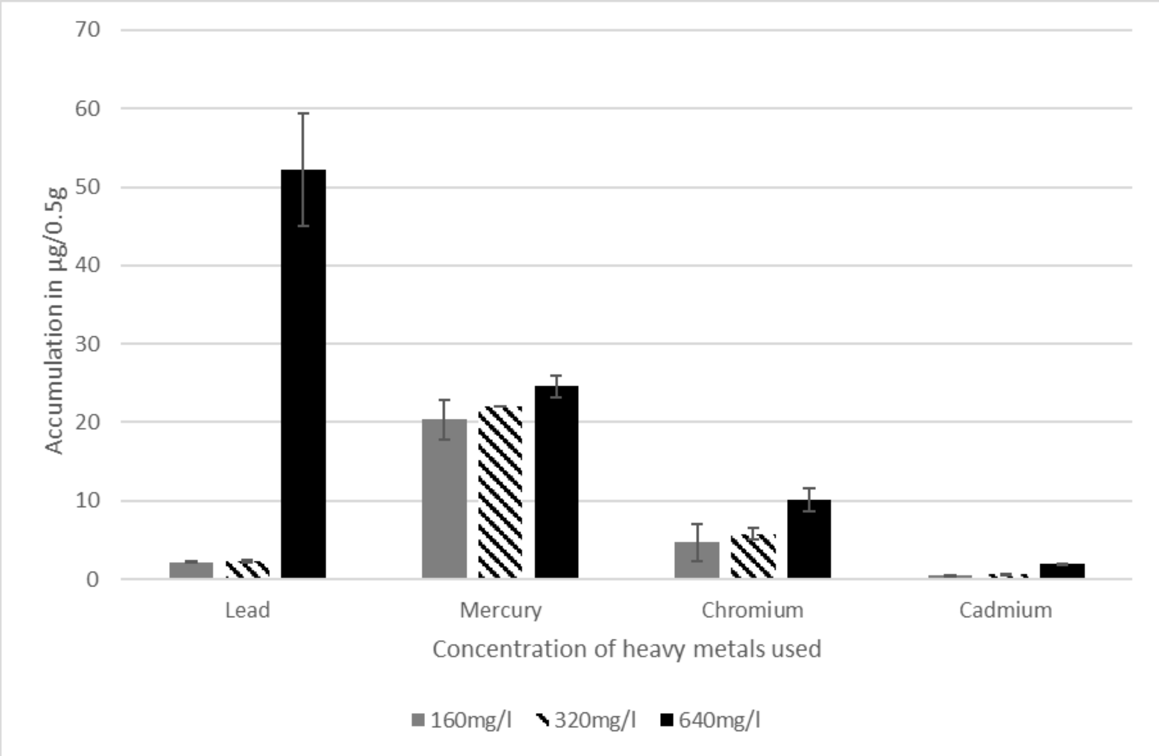

Figure 1- graph showing the heavy metal accumulation in earthworm tissue after two weeks of incubation.

GRAPH REPRESENTING THE HEAVY METAL ACCUMUALTION IN EARTHWORM TISSUE AFTER TWO WEEKS.

The figure 1 concludes that there is an increase in the sequestration of heavy metals in the earthworm tissue with the increase of exposure time. Such studies related to heavy metals and its effects on*Eisenia fetid* has been observed by Spurgeon et al, 1992, Shahmasouri et al, 2005, Srivastava et al, 2004 and Bashiz et al, 2014 [29],[10],[30][31].

In case of lead accumulation in *Eiseniafetida* it has been observed that the accumulation pattern for the treatment concentration 640mg/l has shown an increase with the increasing incubation time. For the treatment concentration of 640mg/l the week 1 accumulation is 11.23µg/0.5g while the week 2 accumulation is 52.166µg/0.5g. Hence the lead accumulation increases with the increasing concentration and incubation time. The increase percentage is estimated to be 364.52%. A study conducted by Spurgeon et al, 1992 [29] showed that exposure of lead is not toxic to *Eiseniafetida*. Study stated that leadwas very well accumulated the tissue of *Einiseniafetida*.

Significant increase in the concentration of mercury in the earthworm tissue was not observed even after two weeks of incubation. Week one accumulation for the concentration 640mg/l is 28.57µg/0.5g while week two was 24.566µg/0.5g. The decrease percent was estimated to be 14.014%. Mercury is highly toxic to *Eiseniafetida* and the decrease in its accumulation could be explained with respect to the toxicity of the metal with*Eiseniafetida*. Such a negative effect of mercury on*Eiseniafetida*has already been conducted byJatwani et al, 2016 [28]. Where they studied the effect of Hg and Co on the protein, lipid and the carbohydrate content in the earthworm tissue. The concentrations used were 0.02, 0.04 and 0.06ppm. The study showed that mercury had more detrimental effects on the earthworm than cobalt and these negative effects increased with the increasing concentration. There was high decrease in the carbohydrate, lipid and protein content in the tissue when treated with 0.06ppm of Hg.

In case of chromium, a dose dependent accumulation of heavy metal was observed. There is a drastic increase in the accumulation of chromium with the increasing time frame. The highest accumulation of chromium in earthworm tissue was seen for the treatment concentration 640mg/l where week 1 accumulation of chromium in earthworm tissue was 4.78µg/0.5g while the week two accumulation was 10.06µg/0.5g. The percentage increase was estimated to be 110.46%. This dose dependent accumulation of chromium has been observed in previous studies done by Shahmasouri et al, 2005 and Bashiz et al, 2014 [10],[31]. The same pattern was observed for cadmium where with the increasing exposure time the amount of metal accumulation increases. For the treatment coancentration 640mg/l the week one accumulation was observed to be 0.627 µg/0.5g while for week two it was observed to be 1.89 µg/0.5g. The percentage increase was estimated to be 202.39%. Such a dose dependent pattern of cadmium accumulation is *Eiseniafetida* has already been observed by Spurgeon et al, 1992 [29]. Where it was studied that cadmium is not very toxic to *Eiseniafetida* and that they are capable of accumulation with the increasing does.

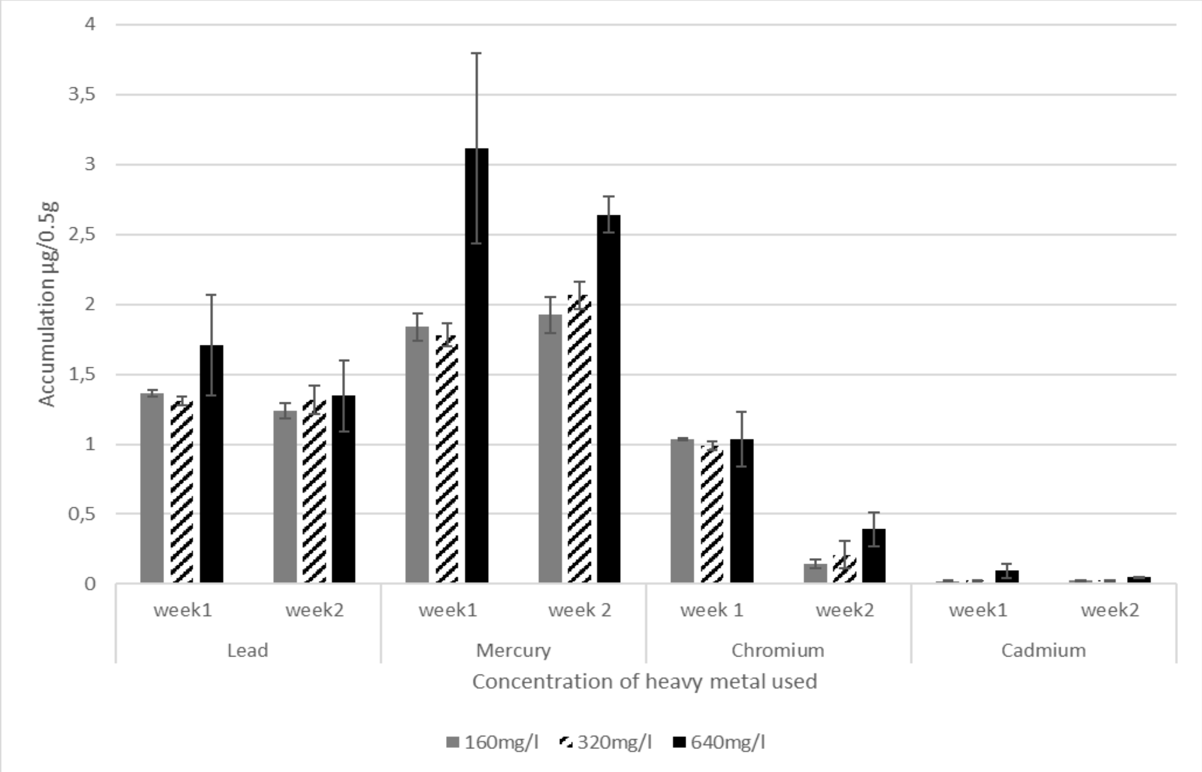

Figure 2 - graph representing the heavy metal present in the soil in plant alone study

WEEK 0 VS WEEK 2 HEAVY METAL ANALYSIS OF SOIL OF ONLY PLANT STUDY.

The figure 2 compares the amount of heavy metal in the initial soil and the soil sample incubated with *Solanumlycopersicum* for two weeks. The general trend to be observed is that of the decreasing amount of heavy metals from the soil with the increasing time frame of incubation with *Solanumlycopersicum.*

There is little removal of lead from the soil, the week 0 value of lead in the soil is 1.705µg/0.5g while the week 2 value was observed to be 1.345 µg/0.5g for the treatment concentration of 640mg/l. The percentage decrease is estimated to be 21.44%. This could be due to the reason that lead is toxic to tomato plant due to which there was less removal of the metal from the soil. The toxic effects of lead have been studied by Akinci et al, 2010 [24].

For the metal mercury for the treatment concentration of 640mg/l the week 0 and week 2 values are 3.113 µg/0.5g and 2.264 µg/0.5g respectively. The percentage decrease is estimated to be 27.27%.

For the heavy metal cadmium for the treatment concentration of 640mg/l the week 0 and week 2 values are 0.094 µg/0.5g and 0.0481 µg/0.5g respectively with a percentage decrease of 48.82%. From the above data it is understood that there is decrease in the heavy metals in the soil which is incubated with *Solanumlycopersicum.*

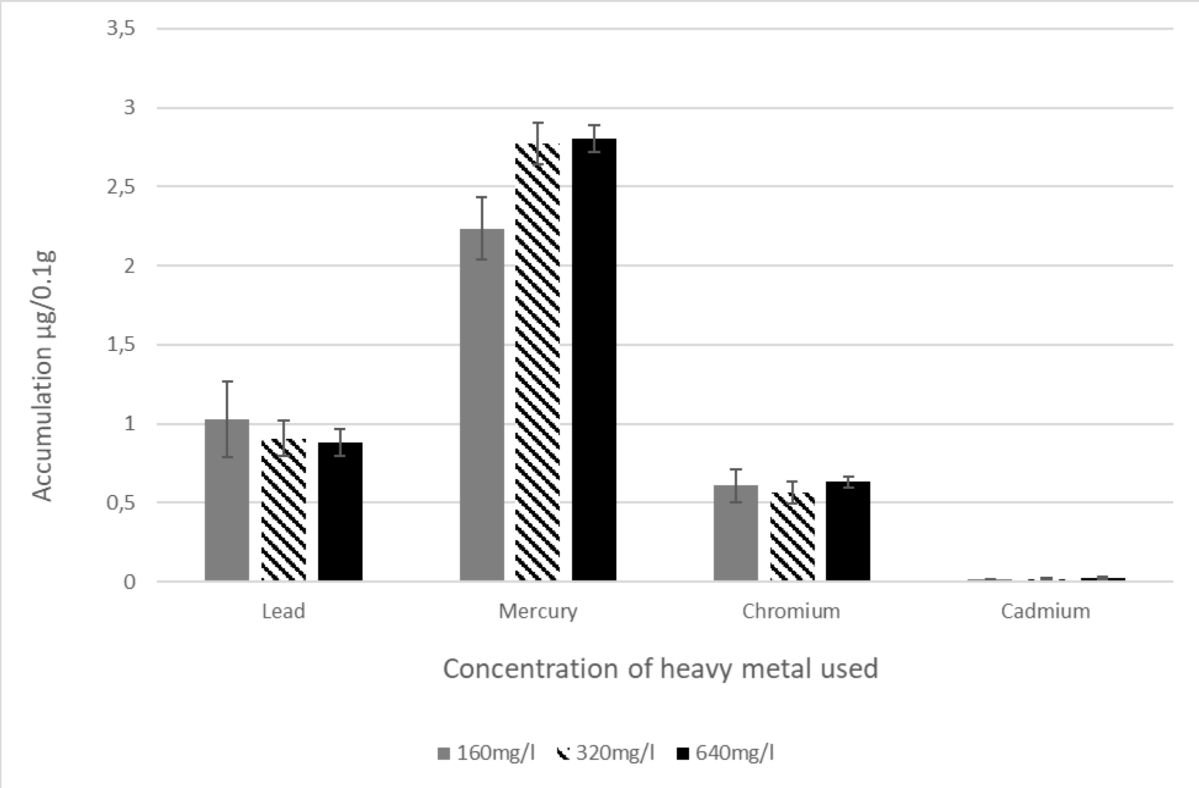

Figure 3- heavy metal accumulation in the leaf of Solanumlycopersicum after two weeks of incubation.

HEAVY METAL ACCUMUALTION IN LEAF TISSUE AFTER TWO WEEKS OF INCUBATION. Plant ALONE STUDY.

From the figure 3, it is observed that *Solanumlycopersicum* is capable of accumulating the heavy metals in a significant amount. In case of lead it has been observed that the plant is capable of accumulating the highest amount for the concentration of 160mg/l which is around 1.030µg/0.1g. As the concentration of the treatment solution increases the accumulation gradually decreases. Similar trend of opposite correlation of accumulation and concentration of treatment have been observed for sapling studies as well. This only proves as *Solanumlycopersicum* is not capable of accumulating lead after a point even if the concentration of the dose is increased. This can be explained with respect to the studies conducted by Akinci et al, 2010 [24]. Where they proved how lead is toxic to *Solanumlycopersicum*and that with the increasing concentration of the treatment solution theaccumulation also gradually decreases.

In case of mercury there is observed to be a gradual increase in the accumulation of mercury with the increasing concentration of treatment solution. For the treatment concentration 160mg/l the accumulation was 2.23µg/0.1g while for the concentration 640mg/l the accumulation is observed to be 2.801 µg/0.1g. There has been a 25.60% increase in the mercury concentration in the leaf of *Solanumlycopersicum*with the increasing treatment concentration.

Chromium shows a gradual increase in the accumulation with the increase in the concentration of treatment solution. For the treatment concentrations 160mg/l and 640mg/l the accumulation is estimated to be 0.60 µg/0.1g and 0.632 µg/0.1g respectively. Showing a 5.33% increase in accumulation. This dose dependent toxicity studies were conducted by Toppi et al, 2014 [25]. Where the concentrations used were 5-10mg/l which did not affect the germination of the seed on vegetables like maize, tomato and cauliflower. In case of tomato there was no formation of phytochelatins in the roots as well as the leaves after treatment with chromium. This could explain why there is a dose dependent increase of chromium.

Similarly, this dose dependent increase in the accumulation is also observed for cadmium. For the treatment concentrations of 160mg/l and 640mg/l the accumulation has been observed to be 0.0138 µg/0.1g and 0.0251 µg/0.1g respectively. The percentage increase is calculated to be 81.88%. Studies conducted by Mediouni et al, 2006, where toxicity of copper and cadmium were studied on tomato saplings. Their study concluded that copper was more toxic than cadmium [27]. Hence a dose dependent increase of cadmium can be observed in the leaf of *Solanumlycopersicum*in our study due its less toxicity to the plant.

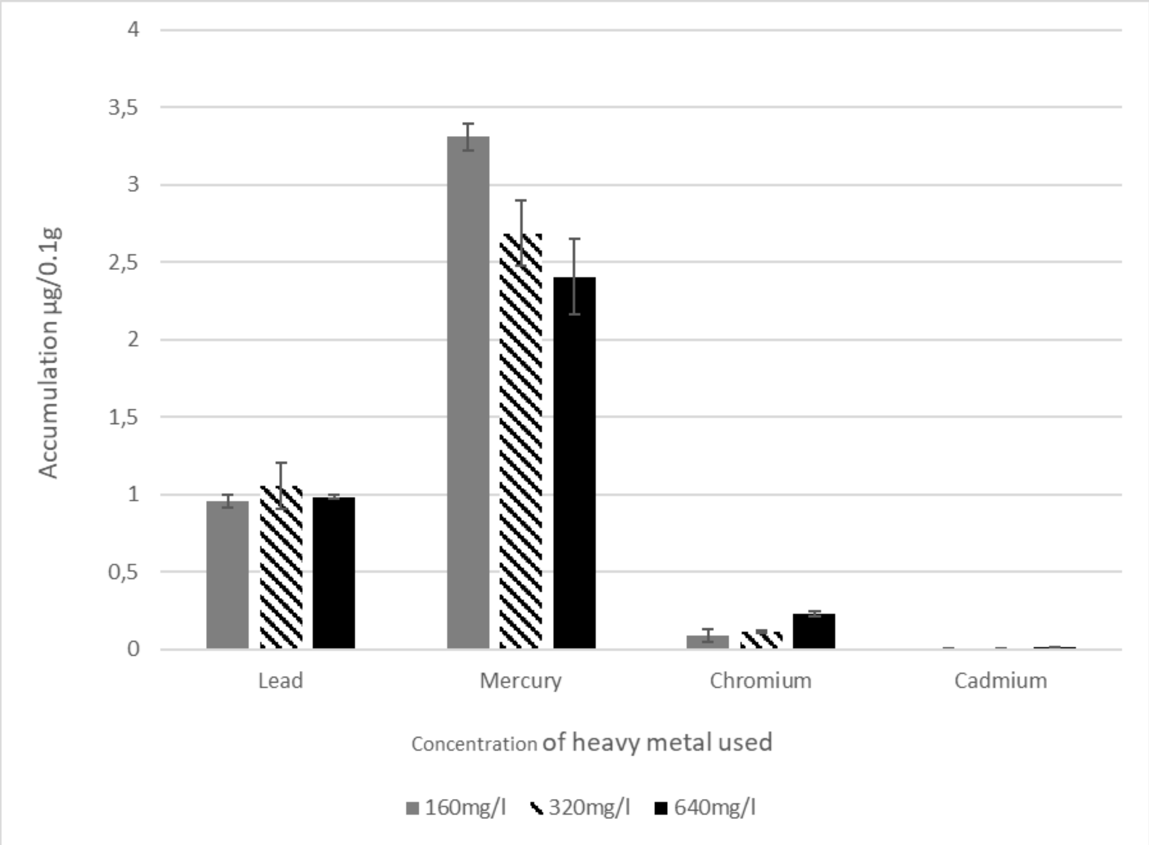

Figure 4 - leaf analysis in the combination study

WORM + PLANT STUDY LEAF ANALYSIS OF SOLANUM LYCOPERSICUM

From the figure 4, it is observed that *Solanumlycopersicum* is capable of accumulating the heavy metals in a significant amount. In case of lead it has been observed that the plant is capable of accumulating the highest amount for the treatment concentration of 160mg/l which is around 0.9575µg/0.1g. As the concentration of the treatment solution increases the accumulation gradually decreases. Similar trend of opposite correlation of accumulation and concentration of treatment have been observed for sapling studies as well. This only proves as *Solanumlycopersicum* is not capable of accumulating lead after a point even if the concentration of the dose is increased. The negative correlation of treatment dosage and accumulation can be explained with respect to the studies conducted by Akinci et al, 2010 [24]. Where they proved how lead is toxic to *Solanumlycopersicum*which can explain why the increasing concentration of the treatment solution, theaccumulation also gradually decreases. In case of mercury there is observed to be a negative correlation. The highest accumulation of mercury in the leaf has been observed for the treatment concentration of 160mg/l which is 3.311µg/0.1g while for the treatment concentration 640mg/l the accumulation in the leaf is estimated to be 2.40 µg/0.1g. Here the percentage decrease is estimated to be 27.514%.

Studies have already been conducted by Toppi et al, 2014 [25] that observes the short term response of chromium by *Solanumlycopersicum*and several other vegetable seeds. Chromium shows a gradual increase in the accumulation with the increase inthe concentration of treatment solution. For the treatment concentration 160mg/l and 640mg/l the accumulation in the leaf is estimated to be 0.089 µg/0.1g and 0.230 µg/0.1g respectively. With the increase percentage of 158.427%.

Similarly, this dose dependent increase in the accumulation is also observed for cadmium. Such studies involving the toxicity of cadmium has been done by Mediouni et al, 2006 [27]. They studied the toxic effects of cadmium and copper on tomato and have observed a dose dependent accumulation of cadmium copper in tomato.In our study for the treatment concentration of 160mg/l and 640mg/l the accumulation have been observed to be 0.00541 µg/0.1g and 0.0125 µg/0.1g giving a percentage increase of 131.05%.

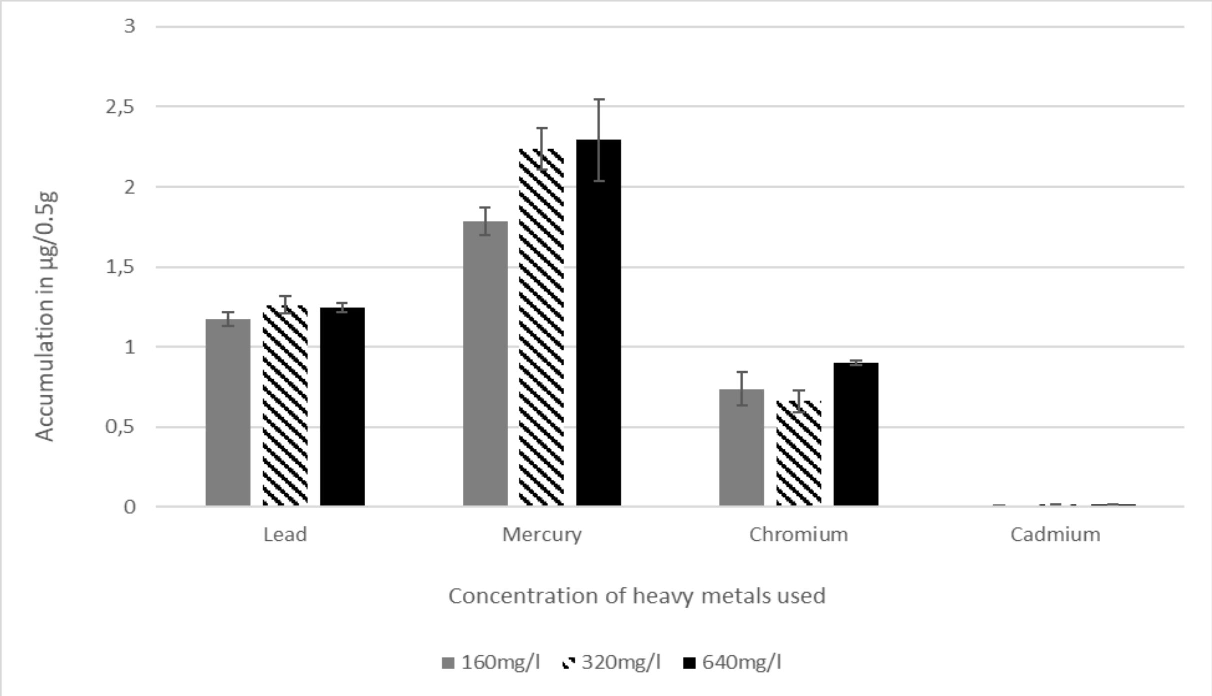

Figure 5 - heavy metal accumulation in Eiseniafetida in combination study.

WORM + PLANT STUDY --- EARTH WORM TISSUE ANALYSIS

The above graph figure 5, concludes that there is an increase in the sequestration of heavy metals in the tissue of *Eisenia fetid* with the increase of exposure time.In case of lead accumulation in *Eiseniafetida* it has been observed that the accumulation of lead in the tissue does not show much increase with the increasing concentration of the treatment. There wasn't much drastic change in the amount of lead accumulated with the increasing does when incubate for two weeks.

For the metal mercury there was observed to be a slight increase in the amount of mercury accumulated in the tissue with the increasing dosage. For the treatment concentration 160mg/l the accumulation is estimated to be 1.1738 µg/0.5g while for the treatment concentration of 640mg/l it is 2.292 µg/0.5g. The percentage increase is estimated to be 95.26%. But for the treatment concentration 320mg/l and 640mg/l the accumulation is only slightly higher. This could be explained due to the fact that mercury is toxic to earthworms this toxicity studies have been conducted by Jatwaniet al, 2016 [28].

In case of chromium there is observed to be a dose dependent accumulation of heavy metal. For the treatment concentrations 160mg/l and 640mg/l the accumulation is estimated to be 0.738µg/0.5g and 0.897 µg/0.5g respectively. The percentage of increase is estimated to be 21.54%. This dose dependent accumulation of chromium has been observed in previous studies done by Shahmansouri et al, 2005[10]. The same pattern was observed for cadmium where with the increasing exposure time the amount of accumulation increases. For the treatment concentrations 160mg/l and 640mg/l the accumulation is estimated to be 0.0084 µg/0.5g and 0.016 µg/0.5g respectively. The percentage increase in estimated to be 90.47%

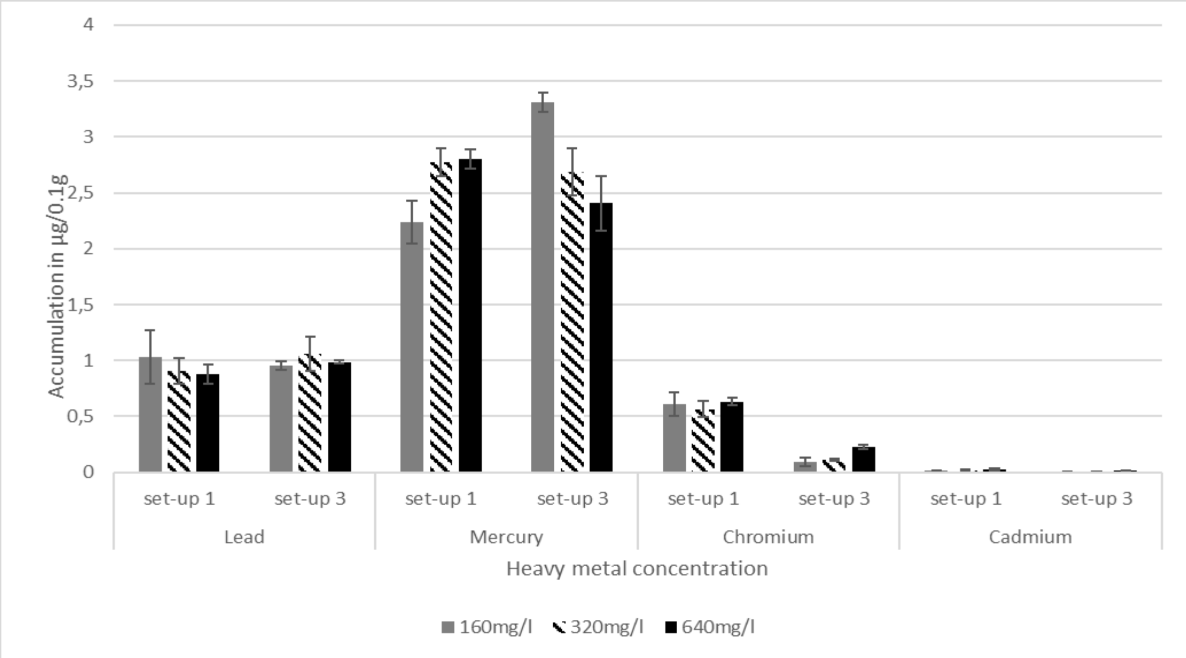

Figure 6 - leaf analysis of set up 1 vs set up 3.

LEAF ANALYSIS OF TOMATO PLANT IN PLANT ALONE VS COMBINED STUDY

Figure 6, compares the amount of heavy metal accumulated in the leaf of *Solanumlycopersicum*after two week of treatment in two different set-ups. Set-up 1 containson*Solanumlycopersicum* while set-up 3 contains both *Solanumlycopersicum* and *Eiseniafetida.* The above data is to understand the ability of*Solanumlycopersicum*toaccumulatedheavy metals with respect to the presence or absence of *Eiseniafetida.*

For the heavy metal lead the pattern observed is that the leaf sample of *Solanumlycopersicum* of the set-up 1 has accumulated less lead in comparison to the leaf sample of *Solanumlycopersicum*of the set-up 3. Studies conducted by Jusselme et al, 2012 [18] have suggested the change in the efficiency of *Solanumlycopersicum* in the accumulation of heavy metal in the presence of Eiseni*afetida.* This can explain the higher accumulation of lead by *Solanumlycopersicum*in the combination studies than the plant alone study.

For the heavy metal mercury, the pattern observed is that the leaf sample of *Solanumlycopersicum*of the set-up 1 has accumulated less mercury in comparison to the leaf sampleof*Solanumlycopersicum* of the set-up 3. For the heavy metal chromium, the pattern observed is that the leaf sample of *Solanumlycopersicum* of the set-up 1 has accumulated more chromium in comparison to the leaf sample of *Solanumlycopersicum* of the set-up 3. Form the figure 6, it is evident that the accumulation of lead and mercury by *Solanumlycopersicum*is affected by the presence of*Eiseniafetida.* Where the presence of*Eiseniafetida*is responsible for the more accumation of lead and mercury in set-up 3. Whileon the other hand for metals such as chromium and cadmium leaf samples from the set-up 1 have accumulated more in amount due to the fact that the set-up 1 one only has plant. As discussed in the previous graphs it was evident that *Eiseniafetida* have more affinity to chromium and cadmium due to which less amount is found in the plant sample of set-up 3.

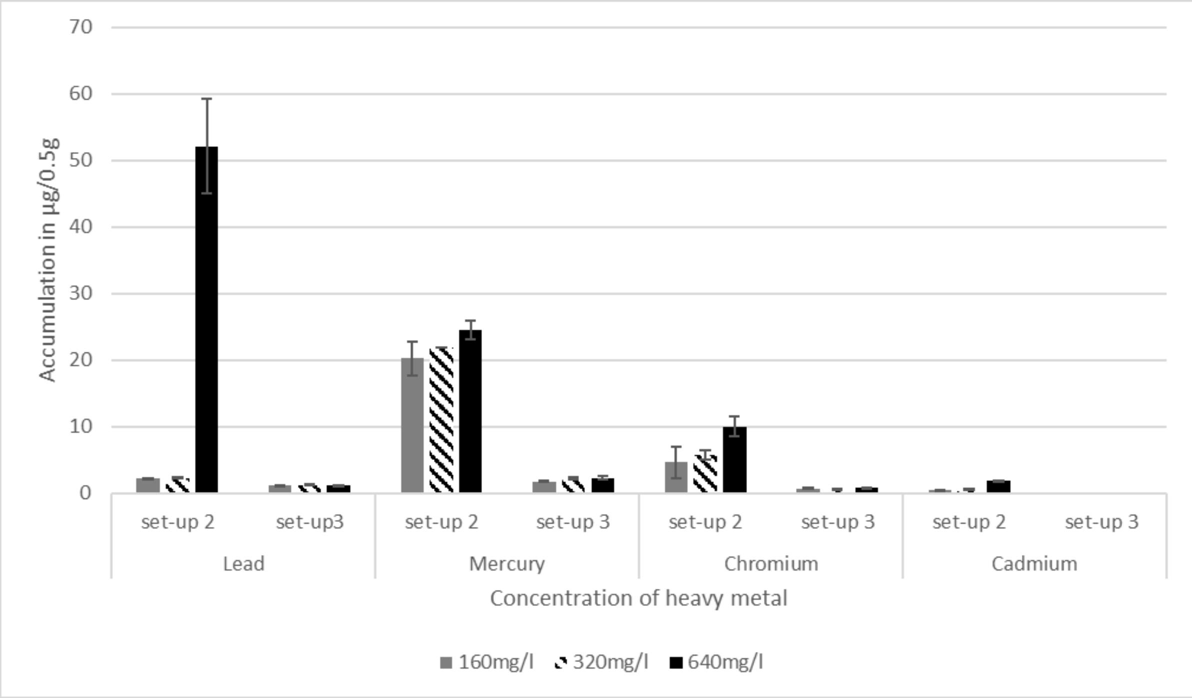

Figure 7 - worm tissue analysis in set up 2 vs set up 3

WORM TISSUE ANALYSIS IN WORM ALONE STUDY AND COMBINED STUDY

For the accumulation of worm alone study only *Eiseniafetida* was exposed to contaminated soil. While the combination study consists of both *Solanumlycopersicum* and *Eiseniafetida*. The above figure 7, compare the amount of heavy metals accumulated by *Eiseniafetida*in both the set-ups.

There is not much difference in the accumulation of lead in both the setups. In case of mercury the worm place in alone (set up- 2) in the treated soil accumulated more amount of mercury in comparison to the worm in the combined study (set-up 3). For the metals chromium and cadmium setup 2 have accumulated more heavy metal than set – 3 comprising both *Solanumlycopersicum* as well as *Eiseniafetida*.

From the above data it can be concluded that there is more accumulation in the tissue of *Eiseniafetida*when it is present alone without*Solanumlycopersicum*.

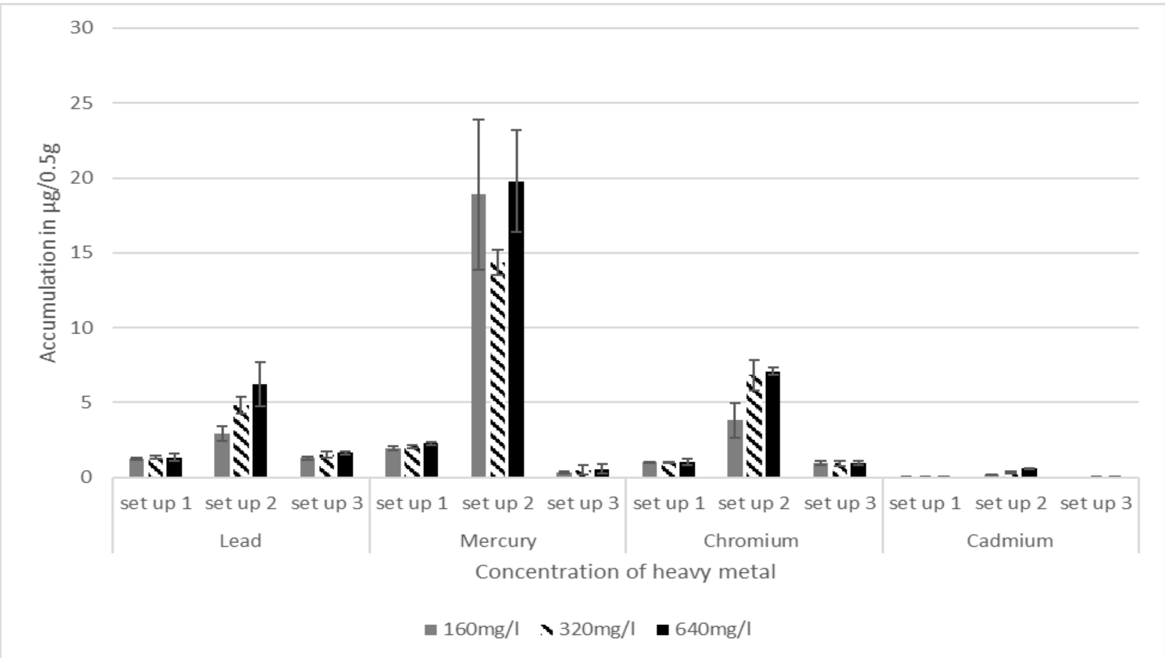

Figure 8 - graph comparing the heavy metal in soil of all the three set ups.

GRAPH COMPARIN FINAL SOIL IN ALL THREE SET UPS.

The figure 8, compares the heavy metals that have been bio remediated from the soil with respect to three set-ups. The first is the *Solanumlycopersicum* alone (set-up 1), the second is *Eiseniafetida* alone (setup 2) while the third one is having both *Solanumlycopersicum*and*Eiseniafetida* together (setup 3). It can be understood that highest remediation of metal has been observed in the combined set-up having both *Solanumlycopersicum* and *Eiseniafetida* together. This trend of bioremediation of soil with plant and worm have been conducted previously by Jusselme et al, 2012 [18] with *Lantana camara* and earthworm. While Macci et al, 2012 [19] have studied such bioremediation studies with tomato plant and earthworm supported by organic matter. Studies conducted by Boukairt et al, 2017 [32] have conducted such studies using barley and earthworm. These studies have similar results with our study as the use of both plant and earthworm has been considered more efficient than them being used alone for bioremediation of soil contaminated with heavy metal.

4. Conclusion

It is observed that the efficiency of the *Solanumlycopersicum* and *Eiseniafetida* to accumulate heavy metals got differed with respect to its presence together in the same set-up. The efficiency of *Solanumlycopersicum* to accumulate chromium and cadmium decreased with the presence of *Eiseniafetida* this can be explained with respect to the fact that *Eiseniafetida* has more affinity to the heavy metals chromium and cadmium which has been previously observed by Liu et al,2005 [12] and Shahmasouri et al, 2005 [10] respectively. While on the other hand it has been observed that the efficiency of *Eiseniafetida* to accumulate metals like mercury and lead has decreased with the presence of *Solanumlycopersicum*, this is due to the fact that metals like mercury are toxic to*Eiseniafetida*withrespect to studies conducted by Jatwani et al, 2016 [28]. From the figure 8 it can be concluded that the combination studies containing both *Solanumlycopersicum* and *Eiseniafetida*are better at bioremediation of heavy metal contaminated soil.

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