Heavy Metal Bioaccumulation by *Eisenia fetida*, *Cynodon dactylon* and *Vigna radiata* in Single, Bi and Tri-Metal Soil Systems

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***Abstract*—Rapid industrialization and consequent anthropogenic activities has magnified heavy metal pollution in the environment as whole, especially in soil systems. It has enhanced the risk of entering of heavy metals in food chain resulting in aggravated health hazards in the higher bios including humans, which has become a serious environmental concern. Additionally, the metal causing soil contamination may be redistributed regionally and globally in other environmental segments with consequent environmental hazard. Though bioremediation techniques including phytoremediation, employing bioaccumulative nature of metals are favored by scientists but heighten the health risks on passing through food-webs. The present short-term study evaluates risks of metal bioaccumulation while soil is decontaminated through sole phytoremediation or integrated with vermicomposting technology. The bioaccumulation experiments were designed with an edible crop-*Vigna radiata*, a fodder species-*Cynodon dactylon* and a soil building worm species *Eisenia fetida* for the short exposure periods of 7 and 14 days. Soils were spiked either with each of lead, cobalt and nickel alone or in combination of two or all three metals. The results show positive risk correlation between metals accumulation in considered biospecies employed for soil decontamination.**

***Index Terms* Heavy Metals, Soil pollution, Bioaccumulation, Phytoremediation, Vermicomposting, Phytovermicomposting**

I. INTRODUCTION

The environment is bearing the adverse overload of the impacts inflicted upon it by the developmental interventions generating various waste streams. Soil, a complex heterogeneous system, interrelates with hydrosphere and atmosphere; thus translocates contaminants to water and air [1]. Heavy metals, extracted through mining, employed in various industrial processes, are prime constituents of industrial wastes that jeopardize the environment enormously and soil in particular. Metal contaminations hamper the functioning of soil ecosystem qualitatively and quantitatively by disturbing the activities of soil organisms [2], and finally degenerate the soil resulting in a sharp decrease in its productivity [3]. Under acidic conditions, metals are more mobile in soil system and easily washout with surface runoff to surface water or seep into groundwater, causing serious hazards. According to ATSDR, nickel released into the environment, ends up in soil or sediment strongly attached to particles containing iron or manganese. Soils near ore deposits, phosphate rocks, or ore smelting facilities, and contaminated by airport traffic, highway traffic, or other Industrial pollution may contain high concentrations of cobalt.

Earthworms play key role in soil formation, maintenance and are sensitive to pollutants, thus used as bioindicators to evaluate soil health and contamination [4, 5 and 6]. Though mature earthworms can store high metal concentrations in non-toxic forms [7], but poses risk of passing through food-webs if not properly confined or consumed by else. Thus, bioaccumulative studies of earthworm become important to evaluate the accumulative nature of metals and their non-degradable properties in order to access the impact of metal bioaccumulation in organism and its more severe effect on the various ecological webs that exist [5]. *Cynodon Dactylon* has been reported to accumulate and stabilize lead. Lead in plants was observed to decrease by 40 folds (remediated soil) and 13 folds (Non-remediated soil) in a period of 10 years [8]. Cynodon dactylon accumulates metal both in roots and shoots [9]. Manganese was found to be accumulated in the root (63 mg/kg) and shoot (36 mg/kg) of Cynodon growing in natural top soil [10]. *Cynodon dactylon* has also been studied for rehabilitation of mine tailings and have been found to accumulate Mn (232.5mg/kg), Pb(5.7mg/kg) and Co(1.9mg/kg) at site[11]. Thus, the commonly found fodder species is an effective accumulator of metal and shows accumulation of metal when exposed to stress, thus indicating the extent of soil pollution.*Vigna radiata*, commonly known as moong dal, is one of the most widely used pulse crops in India. It is highly priced; rich in phosphoric acid and grown in the area adjoining the industries where industrial effluents contaminated with heavy metals is discharged where it acts as an effective bioindicator species. It develops dense pigmentation and also distortion of cells of various tissues takes place due to metal interference with the cell division or with cell elongation.

In this study, controlled laboratory studies were conducted with earthworms reared in soils artificially spiked with single, bi and tri metal combination heavy metal salts ($PbNO_3$, $CoCl_2$ and $NiCl_2$) and plants in case of bi and multi metal systems in order to analyze the accumulation of metal in worms.

II. MATERIALS AND METHODS

A. Collection and Acclimatization of Worms

The earthworm species chosen was *Eisenia fetida,* an epigeic species, recommended by Edwards and Coulson (1992) as the standard laboratory species for eco-toxicological screening i.e. the OECD guideline 207. The earthworms were collected from CMC Vermicomposting Unit, Bagayam, 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.

Cynodon dactylon was brought from VIT University lawns. Roots were washed first in tap water then in de-ionized water to ensure complete removal of bounded soil particles. Plantlets were separated by cutting stolon. Roots and shoots of plantlets were trimmed into equal sizes of 3 cm and 10 cm respectively.

Seeds of *Vigna radiata,* commonly known as moong dal were kept in 1 percent NaCl solution overnight and then transferred to a damp muslin cloth for pre-germination. The sprouts were then sown in the artificially contaminated soil.

B. Experimental Setup

The investigation was carried out in laboratory conditions. The experiments were conducted in polyethylene culture pots, each of capacity 3L. Sandy loam, sieved soil (2.36mm), taken from VIT nursery was mixed with sand in the ratio of 3:1. 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, 2kg of soil and 300g of feed was layered.

To obtain experimental soil beds containing 75, 150, 300, 600 and 800 mg of Pb, 75, 150, 300, 500 and 800 mg of Ni and 10, 20, 40, 60 and 100 mg of Co kg^{-1} soil (Table I), the soil was treated by adding appropriate amount of lead nitrate, cobaltous chloride and nickel chloride made in 300mL of distilled water. 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 ± 2 ºC 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 (10 per bin) were inoculated in each experimental bin in duplicates along with the control beds.

Two exposure periods were tested: 7 and 14 days, and for each duration and dose condition, two replicates, consisting of 10 worms pooled together, were analyzed.

TABLE I.
CONCENTRATION OF METALS FOR SOIL CONTAMINATION

Concentration	Pb (mg/kg)	Ni (mg/kg)	Co (mg/kg)
C1	75	75	10
C2	150	150	20
C3	300	300	40
C4	600	500	60
C5	800	800	100

C. Analytical Procedure

The soil samples were air-dried and ground before chemical analysis. Soil samples were acid digested by USEPA 3050b method and were measured by atomic absorption spectrometer to determine total metal concentrations. To find out the metal concentration in wet body mass, earthworms were kept overnight on a damp filter paper without food for removing their gut contents and sacrificed by keeping in oven at 50 ± 5ºC for 48 hours after which they were digested by USEPA 3050b method for acid digestion. Bioaccumulated metal concentrations were determined by atomic absorption spectrometer. Plant samples were thoroughly washed after dismantling procedure and kept in oven at 50 ± 5ºC for 5 days, crushed and sieved through 1mm sieve, acid digested and analyzed for presence of heavy metals in similar way as earthworms.

III. RESULTS AND DISCUSSION

A. Soil and Feed Background

Background analysis of feed and soil was done in order to determine the pH, moisture content and concentrations of lead, cobalt and nickel already present in soil. The background soil was found to have lead but no trace of cobalt and nickel was detected by analysis from AAS. The soil used for experimentation had very low moisture content and was slightly alkaline.

TABLE II.
BACKGROUND DETAILS OF SOIL AND FEED

Parameters	Soil	Feed
pH	8.76	8.87
Moisture Content (%)	4.5	72
Lead (mg/kg)	27	0
Cobalt (mg/kg)	0	0
Nickel (mg/kg)	0	0

B. Metal Accumulation in Eisenia fetida, Cynodon dctylon and Vigna radita.

Accumulation of heavy metal in earthworms depends strongly on the metal that is bioavailable for uptake rather than the total. A positive biouptake of metal is observed in *Eisenia fetida, Cynodon dactylon* and *Vigna radiata* according to the results obtained.

B.1 Accumulation in Mono-metal system

Soils contaminated with varying single metal concentrations are analyzed in order to observe the stress and bioaccumulation in Eisenia fetida and also to assess the hazardous affects of heavy metals individually on an organism.

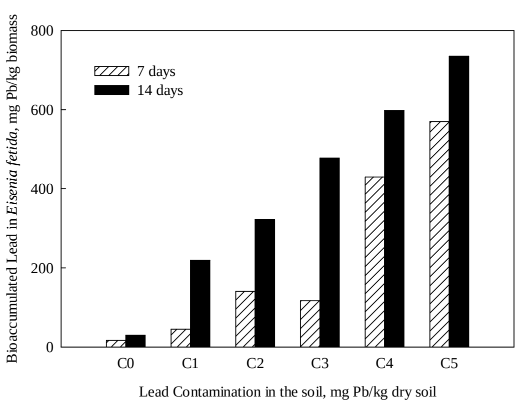

Figure 1. Bioaccumulation of lead in *Eisenia fetida* inoculated for 7 and 14 days in soil contaminated with varying lead concentrations

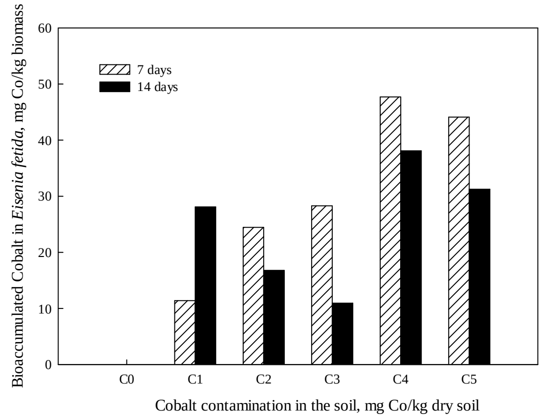

Figure 2. Bioaccumulation of Cobalt in Eisenia fetida inoculated for 7 and 14 days in soil contaminated with varying cobalt concentrations

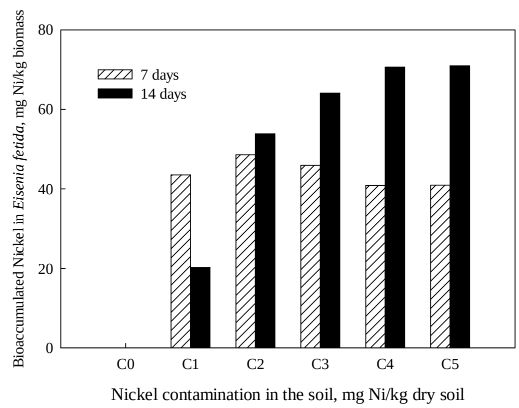

Figure 3. Bioaccumulation of nickel in Eisenia fetida inoculated for 7 and 14 days in soil contaminated with varying nickel concentrations

B.2 Accumulation in Bi-metal system

Bi metal systems are designed to determine the synergistic effect of toxic metals on different biospecies at varying concentrations. In addition to toxicity, interference between metals could be visualized in terms of transfer of metals in different biomaterials. Also many mine tailings are contaminated with two or more than two metals. Thus performance of this integrated technology to remediate such contaminated sites could be assessed. Lead and Cobalt are both toxic and widely studied for accumulation in biospecies, are employed in this study to access. Their accumulation in both the species is evaluated.

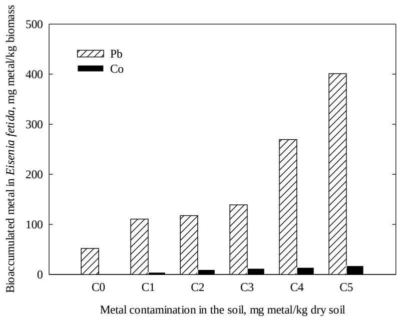

Figure 4. Bioaccumulation of metal in Eisenia fetida inoculated for 7 days in soil contaminated with varying metal concentrations

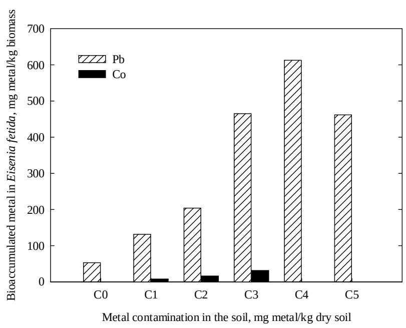

Figure 5. Bioaccumulation of metal in Eisenia fetida inoculated for 14 days in soil contaminated with varying metal concentrations

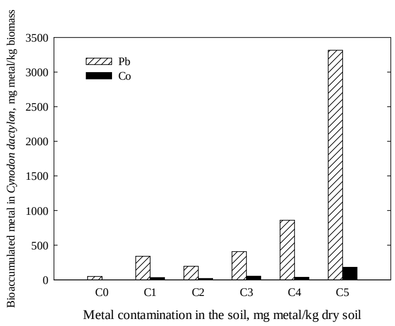

Figure 6. Bioaccumulation of metal in Cynodon dactylon inoculated for 7 days in soil contaminated with varying metal concentrations

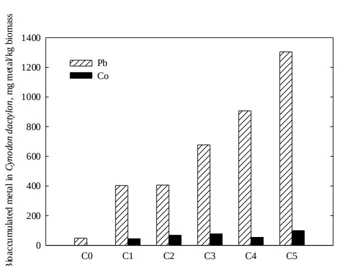

Figure 7. Bioaccumulation of metal in Cynodon dactylon inoculated for 14 days in soil contaminated with varying metal concentrations

B.3 Accumulation in Tri-metal system

A novel approach is carried out to study the extent of decontamination and detoxification of tri-metal contaminated soil using combination of plant and biospecies to find a sustainable remediation solution. The results show an effective accumulation of heavy metals by plant and earthworms both. In *Vigna radiata* stress conditions were observed such as alteration in leaf length, root and shoot length. Thus, can be used as a potential indicator of soil pollution.

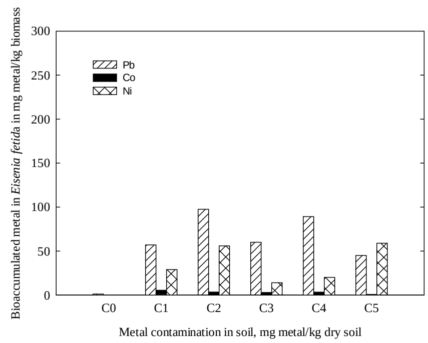

Figure 8. Bioaccumulation of metal in Eisenia fetida inoculated for 7 days in soil contaminated with varying metal concentration

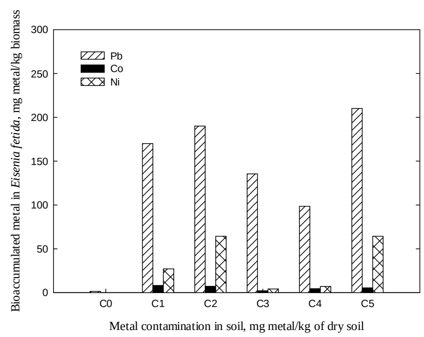

Figure 9. Bioaccumulation of metal in Eisenia fetida inoculated for 14 days in soil contaminated with varying metal concentration

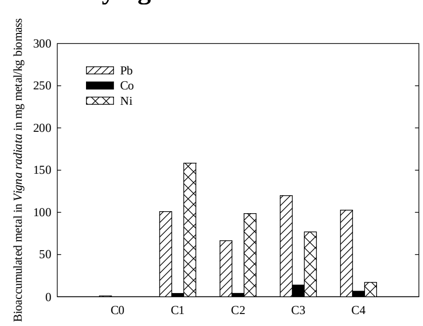

Figure 10. Bioaccumulation of metal in Vigna radiata inoculated for 7days in soil contaminated with varying metal concentrations

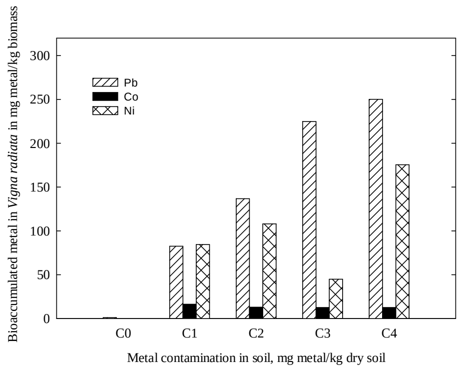

Figure 11. Bioaccumulation of metal in Vigna radiata inoculated for 7days in soil contaminated with varying metal concentrations

IV. CONCLUSIONS

During the experimental period, a positive accumulation pattern was observed in *Eisenia fetida*, *Cynodon dactylon* and *Vigna radiata*. An increased accumulation of lead in *Eisenia fetida* with an increase of exposure period was observed in both single, bi and tri metal contaminated soils, thus earthworms have an efficient lead storage mechanism as they possess Pb nodules. In case of single metal systems, lead and nickel uptake is highest for 14 day exposure period as compared to 7 days, but maximum cobalt is taken by the earthworms in the 7 day exposure period (Figure 1,2,3and 4).For bi.metal systems, accumulation of Pb positively correlated with concentration and exposure period was obtained. For the 14 day exposure period, a decline in accumulation was observed at highest concentration of lead (Figure 5). The possible reasons behind this may be due to decrease in bioavailable metal after 14 day exposure period. Also, Cobalt could have interfered with lead availability to worms. Cobalt accumulation also increased with increase in concentration and exposure period, but at higher concentrations, after 14 days, no accumulation of Co was observed (Figure 5). There was a significant difference in Pb and Co concentrations in soil which may have lead to unavailability of Co to worms. After 7 days accumulation was less or nil at low concentrations of Co (Figure 4). *Cynodon dactylon* accumulated lead and cobalt but after 7 days, accumulation of Pb was higher as compared to 14 days. Initial exposure may have caused sudden uptake owing to the high concentration of lead. *Cynodon dactylon* accumulated cobalt more than earthworm in both the exposure periods (Figure 6, 7). This shows that even at higher concentration of lead, accumulation of cobalt in plant is not interfered as it was the case with earthworms. For tri-metal soil systems, as compared to *Eisenia fetida* plant species, *Vigna radiata* had more metal uptake for 7 days of exposure time (Figure 8, 10), whereas after 14days days earthworms showed better metal accumulation (Figure 9, 11). Out of all the metals lead accumulation was higher and reached up to 250 mg/kg for 14 days exposure time by both the bio-species. The trend of metal uptake is seen higher in 14 days of exposure time for all the metals. However some irregularities indicate that in a multi-metal contaminated soil system presence of one metal suppresses the activity of other metal.

Thus, vermicomposting and integrated phyto-vermicomposting prove the fact that accumulation of metal in plants and organism depends upon the bioavailable metal present in soil for uptake. Metals, non-degradable in nature, are easily uptaken by the biospecies and accumulate, thus posing a severe risk to the environment as they may enter inside the existing food chains and webs. *Eisenia fetida*, *Cynodon dactylon* and *Vigna radiata* can be used as effective indicators of soil health as they show positive accumulation results when exposed to heavily contaminated soils.

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