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PHYTOREMEDIATION: THE APPLICATION OF VERMICOMPOST TO REMOVE ZINC, CADMIUM, COPPER, NICKEL AND LEAD BY SUNFLOWER PLANT

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Abstract

A greenhouse experiment was conducted to determine the phytotoxic effect of heavy metals such as Cd, Cu, Ni, Pb and Zn on the growth of Sunflower (*Helianthus annuus*): on the seed germination, root/shoot growth and uptake of metals in soil-vermicompost media. The selected metals were dosed at various concentrations ranging from 0, 5, 10, 20, 40 and 50 ppm separately in soil - vermicompost media (3:1) in pot experiment. The seed germination, root and shoot growth were found significantly affected by these metals at higher concentration of 40 and 50 ppm. However, the lower concentration of heavy metals ranging from 5 to 20 ppm doses were observed to be stimulating the root and shoot length and increase biomass of the sunflower plant. Sunflower was able to germinate and grow efficiently at all Zn concentration evaluated in this study. The research study of the sunflower indicates the heavy metal uptake at the concentrations 5, 10, 20, 40 and 50 ppm. Sunflower is a very fast-growing industrial oil crop with a high biomass producing plant to be used for phytoremediation (uptake) of toxic metals (Cu, Zn, Pb, Hg, As, Cd, Ni) from soil in heavily contaminated areas. Vermicompost can be used to remediate metals –contaminated sites because it binds metals and increase uptake by providing nutrients such as sodium, magnesium, iron, zinc, manganese and copper which can serve as a natural fertilizer giving high yield of biomass and microbial consortium helped the overall growth of the sunflower plant. The use of vermicompost amended soil would be effective to remediate the heavy metals from contaminated environment.

Keywords: heavy metals, *Helianthus annuus,* phytotoxicity, remediation uptake

1. Introduction

Heavy metal toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems of our modern society. Primary sources of pollution is from the burning of fossil fuels, mining and melting of metallic ferous ores, municipal wastes, fertilizers, pesticides, and sewage sludge (Peng et al., 2006; Xiong, 1998). The most common heavy metal contaminants are: Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Nickel (Ni) and Zinc (Zn) (Lasat et al., 2001; USEPA, 1997).

The term heavy metals refers to metals and metalloids having densities greater than 5 g cm^{-3} and is usually associated with pollution and toxicity although some of these elements (essential metals) are required by organisms at low concentrations (Adriano, 2001). For example, zinc (Zn) is the component of a variety of enzymes (dehydrogenases, proteinases, peptidases) but is also involved in the metabolism of carbohydrates, proteins, phosphate, auxins, in RNA and ribosome formation in plants Mengel and Kirkby, 1982). Copper (Cu) contributes to several physiological processes in plants (photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production) including disease resistance (Kabata-Pendias and Pendias, 2001).

However, at high concentrations, these metals exhibit toxic effects on cells (Baker & Walker, 1989). On the contrary, cadmium (Cd) is not involved in any known biological processes (nonessential metal) and may be quite toxic as it is accumulated by organisms (Peng et al., 2006; Suziki et al., 2001). It is known to disturb enzyme activities, to inhibit the DNA-mediated transformation in microorganisms, to interfere in the symbiosis between microbes and

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plants, as well as to increase plant predisposition to fungal invasion (Kabata-Pendias and Pendias, 2001).

The soil has been traditionally the site for disposal for most of the heavy metal wastes which needs to be treated. Currently, conventional remediation methods of heavy metal contaminated soils include electrokinetical treatment, chemical oxidation or reduction, leaching, solidification, vitrification, excavation, and off-site treatment. These clean up processes of heavy metal pollution are expensive and environmentally destructive (Aboulroos, et al., 2006; Bio-Wise, 2003).

Unlike organic compounds, metals cannot be degraded, and their cleanup requires their immobilization and toxicity reduction or removal. In recent years, scientists and engineers have started to generate cost effective technologies which includes use of microorganisms/ biomass or live plants for cleaning of polluted areas (Qui et al., 2006; Kuzovkina et al, 2004). Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long term applicability (Boonyapookana et al., 2005; Su and Wong, 2004). This technology can be defined as the efficient use of plants to remove, detoxify or immobilize environmental contaminants in a growth matrix (Soil, Water or Sediments) through the natural, biological, chemical or physical activities and processes of the plants (Ciura et al., 2005). It is best applied at the sites with shallow contamination of organic, nutrient or metal pollutants that are amenable to one of the five applications: Phytotransformation, Rhizosphere Bioremediation, Phytostabilization, Phytoextraction, Rhizofiltration (Schnoor, 1997; Yang et al., 2005).

Phytoextraction (uptake) is the use of living green plants in order to remove inorganic contaminants, primarily metals, from polluted soils and concentrate them into roots and easily harvestable shoots (Lasat, 2002; Tang et al., 2003). Phytoremediation can be used to remove not only metals (e.g. Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn) but also radionuclides (e.g. ^{90}Sr, ^{137}Cs, ^{239}Pu, ^{234}U, ^{238}U) and certain organic compounds (Andrade et al., 2002). Plants have shown the capacity to withstand relatively high concentration of contaminants without toxic effects. Some of the heavy metals at low doses are essential micronutrients for plants, but in higher doses, may cause metabolic disorder and growth inhabitation for most of the plant species (Sinha, 2005). Researchers have observed that some plant species are endemic to metallic-ferrous soil and can tolerate greater than the used amount of heavy metal or other compounds (Peralta et al, 2000). Plants such as Indian mustard (*Brassica juncea*), Corn (*Zea mays L.*) or sunflower (*Helianthus annuus* L.) show high tolerance to heavy metals and therefore, are used in phytoremediation studies (Pilon-Smits, 2005; Schmidt, 2003; Tang et al., 2003). The plant material may be used for non-food purposes; alternatively, it can be burned followed by recycling of the metals or as disposal in a landfill (Angel and Linacre, 2005; Bennett et al., 2003). In the present study we examine the potential of sunflower plant for phytoremediation (uptake) of heavy metals which is reported to be the fast-growing deep-rooted industrial (Prasad, 2004) oil crop with a high biomass (Zhuang, 2005) producing plant species to remove heavy metals such as zinc or copper as well as several radionuclides from contaminated environment (Nehnevajova et al., 2005). Ximenez-Embun et al. (2001) also states that sunflower plant is effective in removing Pb, Cr, Zn, Cd and Ni.

The uptake efficiency of the plant depends on soil type, plant species and conditions. Soil receiving repeated application of chemical fertilizers, fungicides, and pesticides have exhibited high concentrations of extractable heavy metals and subsequently resulted in increased heavy metal concentrations (Arthur et al., 2005). It has been observed that prolonged use and lavish application of chemical fertilizers reduce land productivity and crops become dependent on periodic inputs of the chemical fertilizers. The factories manufacturing fertilizers are continuously polluting the air, land and water with the price of the fertilizers increasing every year (Maharashtra Nature Park Socity, 2003). The addition of these fertilizers, however, can also inhibit the uptake of some major metal contaminants, such as Pb, due to metal precipitation as pyromorphite and chloropyromorphite (Chaney et al., 2000). Natural chelating action of plants or microbial origin seem more promising than synthetic chemical chelating agent, since chemical chelating agents have additional toxicity to plants, thus they may increase the uptake of metals but decrease plant growth thus proving to be of limited benefit (Malik et al., 2000).

Phytoremediation has gained popularity with government agencies and industry in the past 10 years. This popularity is based in part on the relatively low cost of phytoremediation. Currently, \$6-8 billion per year is spent for environmental cleanup in the United States, and \$25-50 billion per year worldwide (Pilon- Smits, 2005). This plant based technique is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. Biological processes such as composting followed by vermin-composting to convert vegetable waste (as valuable nutrient source) in agriculturally useful organic fertilizer would be of great benefit. The composting followed by vermin-composting of vegetable waste with earthworm (*Eisenia foetida*) develops in to a natural fertilizer (Maharashtra Nature Park Bulletin, 2003). The vermicompost contain high nutrient value, increases fertility of soil and maintains soil health (Suthar et al., 2005). Application of compost and vermin-compost in contaminated soil improves soil fertility and physical properties as well as helps in successful approach to phytoremediation which has been demonstrated by Zheljazkov and Warman (2004). It also enhances quality of growing plants and increased biomass which could suggest that more metal can be taken up from the contaminated growth media and the tolerance to the metal toxicity is improved (Tang et al., 2003). The use of vermin-compost developed from vegetable waste by vermin-culture biotechnology with soil would provide natural environment for phytoremediation (Elcock and Martens, 1995).

The presented work deals with the application of vermin-compost developed from vegetable waste in soil contaminated with heavy metals for phytoremediation studies. The objectives of this study is to determine the effects of heavy metals on seed germination, plant growth and examine their uptake by sunflower in soil-vermicompost media.

2. Experimental

2.1. Soil sampling, processing, and characterization

Soil was collected from a depth of about 0-15 cm along the banks of Surya River, Palghar (located 100 km away from Mumbai). Stones and plant tissues were carefully removed from the soil prior to drying process under laboratory condition. The soil was screened through 2 mm stainless steel sieve, and stored in a plastic bag at room temperature until use. Concentrations of Pb, Zn, Cu, Ni, and Cd were measured by atomic absorption spectrophotometer (APHA, 1998). The physicochemical parameters were measured by standard methods (Table1). Soil texture was determined by the hydrometer method. The moisture content of soil was calculated by the mass difference before and after drying at 105 ^{0}C to a constant mass. The pH and electrical conductivity (EC) were measured after 20 min of vigorous mixing samples at 1: 2.5 : : solid : deionized water ratio using digital meters [Elico, Model LI-120] with a combination pH electrode and a 1-cm platinum conductivity cell respectively. Total nitrogen and total phosphorus were determined according to the standard methods of the American Public Health Association (1998). Cation exchange capacity was determined after extraction with ammonium acetate at pH 7.0, and the organic carbon was determined by using Walkley–Black method (Jackson, 1973).

2.2. Vermi-composting development

The vermin-compost was produced from vegetable waste (cabbage, french bean, cauliflower, lady finger, spinach, carrot, and radish) collected from the vegetable market. In the process dry leaves, coconut fibers and fresh grasses having high lignin content were taken with the vegetable waste in appropriate quantity. About ½ Kg of exotic varieties of earthworms (*Eisenia foetida*) was spread on bedding materials. Everyday, 200 to 300 g of vegetable waste collected from market was supplied for a period of two and half months as a source of food for the earthworms.

The physicochemical parameters were measured during vermin-composting as described in soil analysis. After two and half month, vermicompost was collected, air dried, sieved (2-mm) and a portion of it was taken for nutrient analysis in order to prove its potency as biofertilizer. The nutrients in dried sample of vermin-compost was digested with concentrated nitric acid and 30% hydrogen peroxide and then determined by an atomic absorption spectrophotometer [AAS, Perkin Elmer] (APHA, 1998).

2.3. Greenhouse experiments

Green house pot culture experiments were conducted to study the effect of heavy metals viz Cd, Ni, Cu, Pb, and Zn on seed germination, root growth, shoot growth and phytoremediation (uptake) by sunflower. The ratio 3:1 of alluvial soil and vermi-compost were used as media. Nutrient and trace elements concentrations in the vermin-compost are presented in table 2. This soil–vermin-compost media was then amended with the heavy metals: Cd as Cd $(NO_3).4H_2O$; Cu as $CuSO_4.5H_2O$; Ni as Ni $(NO_3)_2$; Pb as Pb $(NO_3)_2$ and Zn as Zn $(NO_3)_2.6H_2O$. The concentrations of each heavy metal used in this study were 0, 5, 10, 20, 40 and 50 ppm. Sunflower seeds were obtained from seed supplier Ratanshi Agro-Hortitech (Byculla, Mumbai). Seeds were immersed in 3% (v/v) of formaldehyde for 5 minutes and washed with distilled water several times to avoid fungal contamination. The soil-vermi-compost media contaminated/amended with the heavy metal was used as potting media. To determine the effect of heavy metals; 30 seeds of uniform size were placed in every Petri dish (the lids were left partially open until 3 days after the germination) for study of seed germination. The Petri dish were kept in the dark and observed for germination. The seeds were considered germinated with the emergence of radicles. Small plastic pots/glasses were used for studying shoot and root growth. Ten plants were grown in 2 Kg capacity plastic pots for phytoremediation study. Soil-moisture content was adjusted regularly by mass to about 60% of water-holding capacity with deionized water.

To prevent loss of nutrients and trace elements out of the pots, plastic trays were placed under each pot and the leachets collected were put back in the respective pots. Each treatment of plant consisted of three replicate for statistical purpose. The seeds were set under 12/12 hrs light/dark cycle and temperatures of 30^{0}C during the day and 27^{0}C during the night. The average relative humidity was recorded to be 75%. The seedlings were harvested after two weeks; germination rate and shoot/root length were recorded. For the phytoremediation study plants were harvested after 10 weeks. The plants were then separated into roots and shoots. The plant samples were washed with distilled water and dried in an oven at 70^{o}C for 3 days, and the dry mass of biomass was determined, after which these samples were stored in the brown paper bags. The samples were considered for analysis of metal content digested with concentrated nitric acid and 30% hydrogen peroxide and then the heavy metal content was determined by an atomic absorption spectrophotometer [AAS, Perkin Elmer] (APHA, 1998).

2.4. Statistical analysis

Each treatment for % seed germination, plant root/ shoot growth and uptake consisted of three replicate for statistical purpose. The data presented for each treatment in this study is represented as mean of samples with standard deviation (X + S.D.) calculated by standard statistical methods (Mahajan, 1997).

3. Results and discussion

3.1. Soil analysis

After selecting the probable site, soil was collected and subjected to extensive analysis of various physicochemical parameters, which influence root establishment in soil (Table 1).

Soil, texture, which was found to be sandy loam, had profound effect upon the properties of soil including its water supplying power, rate of water intake, aeration, fertility and ease of tillage. The pH was 7.2, which lies within the recommended value for proper growth and efficient uptake of nutrients and compounds from soil. The percentage of organic matter and nitrogen were found to be 0.80 and 0.05 respectively. Macronutrients including metals were also present in substantial amount. Further to augment the existing native state of soil, it was spiked with vermin-compost, which gives all the nutrients conducive to plant growth for phytoremediation studies. There was no history of heavy metal (Cd, Ni, and Pb) contamination found in the soil collected.

3.2. Vermi-composting analysis

The vermin-compost developed was characterized and found to have high concentration of nutrients such as Zn, Cu, Mg, Fe, and Mn (Table 2). This vermin-compost developed by the vermin-culture biotechnology was then used as a natural fertilizer for phytoremediation studies of heavy metals. Several researchers have demonstrated that earthworm castings (vermicompost) have excellent aeration, porosity, structure, drainage, and moisture-holding capacity. The vermin-compost is a rich source of beneficial microorganisms and nutrients (Paul, 2000) and is used as a soil conditioner or fertilizer (Hattenschwile and Gaser, 2005; Rock and Martnes, 1995). Increase in crop yield, soil nutrients status and nutrients uptake was reported due to application of vermicompost (Roberts et al., 2007; Singh and Sharma, 2003). Experimental work has shown that earthworm activity enhances tree seedling growth associated with enhanced soil organic matter, improved nutrition (including NO^{3-}, NH^{4+}, and Ca^{2+}), and increased mycorrhizal colonization (Welke and Parkinson, 2003).

Table 1. Physicochemical properties of experimental soil. †

Soil parameters	*Values*	*S.D.*
Clay %	25.9	±1.8
Silt %	21.7	±2.5
Sand %	50.4	±2.8
pH	7.2	±0.1
Organic matter %	0.80	±0.1
Nitrogen %	0.05	±0.02
C EC* c mol/ 100 g soil	11.27	±0.76
EC‡ dS^{-1}	1.1	± 0.1
Potassium mg/kg	22.73	±2.63
WHC** %	62	±4.0
Moisture Content %	34	±1.8
Heavy metals ppm		
Cu	3.6	± 0 .5
Cd	ND	
Ni	ND	
Pb	ND	
Zn	12	± 1.5

† Values are averages of three replicates ± S.D.
* Cation exchange capacity
‡ Electrical conductivity.
**Water Holding Capacity

Yield of a tropical leguminous woody shrub, *Leucaena leucocephala*, in amended Pb–Zn mine tailings has been found to be increased by 10 to 30% in the presence of burrowing earthworms (*Pheretima spp.*) (Ma et al., 2003). The consecutive effect of organic treatment on the sunflower root yield caused a considerable growth of root system mass (in comparison with the mineral treatment) in the objects where vermin-compost with added cardboard (by over 28%) and straw (by over 21%) was applied (Gondek and Filipek-Mazur, 2003).

Table 2. Chemical and nutrient status of vermin-compost†

Parameters	*Values*	*S.D.*
pH	6.8	±0.173
EC‡ dS m^{-1}	10.55	±0.01
Total C %	13.5	±0.7
Total N %	1.33	±0.015
Available P %	0.47	±0.09
Sodium mg /100g	354.68	±9.44
Magnesium mg/100g	832.48	±22.48
Iron mg/100g	746.26	±23.39
Zinc mg/100g	16.19	±0.55
Manganese mg/100g	53.86	±2.84
Copper mg/100g	5.16	±0.36

† Values are averages of three replicates ± S.D.
‡ Electrical conductivity.

The earthworms increased available forms of N and P in soil, increased metal bioavailability, and raised metal uptake into plants by 16 to 53%. Some evidence indicates that earthworms increase metal bioavailability in relatively low-level metal-contaminated soils with higher organic matter contents (Rida, 1996). This agrees with results of experiments in which the addition of exogenous humic acid to soil has been shown to increase plant-available metals (Halim et al., 2003).

3.3. Effect of heavy metals on seed germination

The present research demonstrated a concentration dependent inhibition of the seed germination with regards to alfalfa species (Table 3). The results of this study indicated that Cd, Ni and Pb at 5 ppm levels had very low toxic effects on seed germination while copper at the same doses increased seed germination. The most toxic metals for seed germination were Cd, Cu, Ni and Pb at 10, 20, 40 and 50 ppm levels. Delayed germination was also observed in all cases at higher i.e 40 and 50 ppm concentrations.

The resulting rank order of toxicity for metals on seed germination was Cd > Cu > Ni > Pb >Zn. However, seed germination increased at all Zn concentrations. Metal removal from contaminated media started at first stage of the germination process when the seed absorbed water and therefore large quantities of metal ions. Once accumulated, ions enter into the root, where they can be stored or exported to the shoot *via* the transpiration stream (Ximenez-Embun et al., 2001). Claire and coworkers (1991) obtained similar results in a study using nickel and other heavy metals on cabbage, lettuce, millet, radish, turnip, and wheat. The heavy metals (Cd and Ni) at higher concentration inhibit the seed germination and growth of sunflower plant (Khan and Moheman, 2006). The failure of the treated seeds of sunflower to germinate at the high concentrations of the applied $CdCl_2$ may be consequence of retarded water uptake, inhibited cell divisions and enlargements in the embryo and or an overall decrease in metabolic activity relevant to these steps. The blockage of any one of the phases may inhibit the germination process. (Shaddad et al., 1989). In this respect, Bazzaz et al. (1974) demonstrated that interference with stomatal function is a primary mode of action of cadmium and several other heavy metals.

3.4. Effect of heavy metals on root growth

The doses of 5 ppm and 10 ppm of Cd, Ni, Pb and Cu promoted the root growth of the plants. The heavy metals Cu, Ni and Pb at 20 ppm level further increased the root growth over the control root size. However, at the same dose Cd reduced the root growth. Cadmium, nickel, copper and lead significantly reduced root size at 40 and 50 ppm dosages. All the Zn concentrations increased the root length than the control root length of sunflower plants. Increase in the heavy metal concentration in the soil-vermin-compost media caused root length decrease with stunt growth of roots (Table 3). Root toxicity symptom included browning, reduced number of roots hair, and growth. In comparison to the control treatment without heavy metals (Cd, Pb, Cu, and Ni), plant roots were healthy and normal.

The color of the roots receiving higher heavy metals treatment (40 and 50 ppm) except Zn, changed gradually over time from creamy white color to dark brown, an indication of intense suberification. There was reduction in the formation of secondary roots and number of root hairs by cadmium ion at 40 and 50 ppm. Plants treated at lower concentrations were not significantly affected by the metals. All zinc, concentrations showed increase in root growth. Lateral roots were observed in almost all treated samples of Zn, Cd, Cu, Pb and Ni demonstrated concentration dependant inhibition of root growth at higher ppms. One of the explanations for roots to be more responsive to toxic metals in environment might be that roots were the specialized absorptive organs so that they were affected earlier and subjected to accumulation of more heavy metals than any of the other organs This could also be the main reason that root length was usually used as a measure for determining heavy metal- tolerant ability of plant (Xiong 1998).

Nandkumar et al. (1995) demonstrated that root growth of Pb treated sunflower plants was retarded compared to the controls. Boonyapookana et al. (2005) found similar results using *H. annuus* in Pb contaminated soil (2.5mM). According to Chaignon and Hinsinger (2003), higher concentrations of copper can inhibit root growth before shoot growth and can accumulate in the roots without any significant increase in its content of the aerial parts. Previous studies have demonstrated that growth of *T. caerulescens* roots was essentially unaffected by 20 ppm concentration of cadmium relative to the control cultures without cadmium (Boominathan and Doran, 2003). Root density and the depth of rooting are particularly significant in the context of phytoremediation. Shaddad et al., (1989) reported a depressed root elongation in *Zea mays* seedlings in the presence of cadmium.

3.5. Effect of heavy metals on shoot growth

The heavy metals Cd, Cu, Ni, and Pb showed increased shoot growth at 5 and 10 ppm levels. At the 20 ppm concentration Cd reduction was noticed in shoot length against the further increase in shoot length of sunflower plants at 20 ppm of Cu, Pb and Ni contaminations. When the concentration of these metals was increased to 40 and 50 ppm dose, significant reduction in the shoot size of the plants was found as compared to the control plants. All plants grown in the media contaminated with Zn showed increase in the shoot growth than the plants grown in media without Zn contamination. All the plants appeared to be healthy and the shoot growth of the plant was stimulated by a concentration of 5, 10, and 20 ppm by the heavy metals (Table 3).

Table 3. Seed germination, root and shoot length of sunflower after two weeks of exposure to heavy metals†

Metal	*Dose (ppm)*	*Germination rate (%)*	*Root length (cm)*	*Shoot length (cm)*
Cd	0	70 ± 4	9.1 ± 0.50	7.5 ± 0.7
	5	65 ± 4	9.7 ± 0.6	18.1 ± 0.8
	10	60 ± 3	10.5 ± 0.71	20.3 ± 0.82
	20	53 ± 3	8.5 ± 0.31	16.2 ± 0.27
	40	46 ± 6	7.1 ± 0.43	12.6 ± 0.65
	50	40 ± 5	5.8 ± 0.88	10.01 ± 0.74
Cu	5	75 ± 5	10 ± 0.86	17.8 ± 1.88
	10	63 ± 4	11.3 ± 0.91	18.3 ± 0.76
	20	60 ± 7	13.8 ± 0.61	19.5 ± 1.38
	40	55 ± 3.05	8.7 ± 0.2	16.7 ± 1.18
	50	50 ± 6	8.3 ± 0.5	14.4 ± 1.28
Ni	5	66 ± 4	9.8 ± 0.68	17.2 ± 1.39
	10	63 ± 4	10.7 ± 1.58	19.2 ± 0.9
	20	56 ± 3	12.3 ± 1.5	20.2 ± 3.1
	40	48 ± 5	8.4 ± 0.5	17.3 ± 1.7
	50	43 ± 2	7.2 ± 0.62	14.3 ± 1.3
Pb	5	70 ± 7	10.64 ± 1.54	18.3 ± 1.5
	10	57 ± 5.5	11.45 ± 1.15	19.4 ± 1.4
	20	56 ± 3.05	13.2 ± 1.4	20 ± 1.5
	40	60 ± 4	8.6 ± 0.5	14.5 ± 1.19
	50	46 ± 4	7.9 ± 1.2	16.2 ± 1.35
Zn	5	74 ± 5.56	11.9± 1.69	18.5 ± 2.29
	10	76 ± 7	14.3 ± 1.52	19.7 ± 1.4
	20	84 ± 5	15.1 ± 1.3	21.6 ± 2.04
	40	92 ± 7.37	15.8 ± 0.81	22.8 ± 2..25
	50	94 ± 4	17.3 ± 1.47	24.7 ± 2.2

† Values are averages of three replicates ± S.D.

These results indicate that low concentrations of Cd, Cu, Ni, and Pb have micronutrient action effects on the sunflower plants. However, at 40 and 50 ppm gradual reduction of the shoot growth was observed as compared to control shoot growth. These symptoms were more common in case of cadmium treatments.

No signs of toxicity from the high levels of Zn were observed in the shoot. All zinc concentrations showed positive effects and shoot growth efficiently. The Pb treated sunflower (*Helianthous annuus*) plants at higher concentrations showed stunted growth and reduced leaf expansion (Nandkumar et al., 1995). Christodoulakis and Margaris (1996) demonstrated that the sludge from sewage treatment products (domestic activities) increased leaf area in sunflower. Ormrod et al. (1986) noted that nickel caused stunted and deformed growth of shoot with symptoms of chlorosis. Wenger et al. (2003) reported that the critical toxicity level of Cu in the shoots of crop plants is greater than 20 to 30 mg kg^{-1}. These data corresponded with those of Oncel et al. (2000), who found that Cd reduces chlorophyll a and b in wheat. Chatterjee and Chatterjee (2000) determined that Cu significantly decreased the water potential and Fe concentration in cauliflower.

The influence of relatively higher amounts of Cu, Zn, Pb, Ni, Cr and Cd in wheat cv. Vergina resulted in depressed shoot growth (Athar and Ahmad, 2002). Stunted growth and visual toxicity symptom of cadmium in shoot of sunflower plant was demonstrated by Singh (2006).

3.6. Effect of heavy metals on plant biomass

The biomass results after 10 weeks of experiments indicated that the mean plant biomass of sunflower showed increasing tendency as the concentrations increased from 5 to 10 to 20 ppm for Cd, Cu, Ni and Pb (Table 4). Biomass decreased gradually as the concentration of Cd, Cu, Pb and Ni in the soil- vermicompost media increased to 40 and 50 ppm. The biomass yield affected by the higher ppm levels of Cd caused reduction in the plant biomass. There was a significant positive effect seen in all Zn concentrations and increase in biomass yield as compared to control ones. Kayser (2001) demonstrated that *Helianthus annuus* produced the largest biomass as compared to *B. juncea* and S. *viminalis* in Zn and Cd contaminated soil. Boonyapookana et al. (2005) have studied that the plant biomass of *H. annuus* in Pb contaminated nutrient media declined with increasing concentration. Higher doses of heavy metal can affect physiology, reduced plant growth and dry biomass yield (Grifferty and Barrington, 2000; Roy et al., 2005).

Similar findings were evaluated with sunflower in artificially contaminated soil with heavy metals (Zn, Cd, Pb and Cu), Herrero et al., (2003) reported that part of the plant with greatest biomass was the shoot (50% of total mass) and the biomass decreased proportionally to the contamination level. Nwosu, et al. (1995) observed that the mean plant biomass decreased in both lettuce and radish, as the concentration of Cd and Pb in soil increased. Grifferty and Barrington (2000) showed that the increased Zn concentration from 25 to 50 mg/kg had a significantly positive effect on dry biomass yield. The application of Tobacco plant in metal uptake by high biomass-producing plants was mentioned by Wenger et al. (2003). Higher levels of organic matter (882.30g/ kg) and nutrients content in the compost had beneficial influence on soil chemical and biochemical properties and plant growth, thus increasing biomass yields (Yang et al., 2005). The plant biomass may be incinerated either to reduce volume, recover energy, disposed off using appropriate techniques or recycled to recover valuable metals (Angel and Linacre, 2005).

Nehnevajova et al., (2005) noted that *Helianthus annuus* accumulated relatively very low amounts of heavy metals in flowers and sunflower seed and sunflower oil may be used for technical lubricants or energy production. The fertilization with vermin-composts and untreated tannery sludge caused a statistically proved increase in the sunflower shoot yield as compared with the mineral treatment (Gondek and Filipek-Mazur, 2003).

Table 4. Biomass of sunflower after 10 weeks of growth in heavy metals enriched soil-vermi-compost media †

Metal	*Dose (ppm)*	*Root Dry Weight (g)*	*Shoot Dry Weight (g)*
Cd	0	1.510 ± 0.108	2.450 ± 0.138
	5	1.680 ± 0.17	2.490 ± 0.37
	10	1.832 ± 0.124	2.570 ± 0.47
	20	2.01 ± 0.64	2.712 ± 0.293
	40	1.332 ± 0.068	1.906 ± 0.268
	50	0.815 ± 0.08	1.371 ± 0.163
Cu	5	1.619 ± 0.092	2.503 ± 0.114
	10	1.862 ± 0.139	2.735 ± 0.155
	20	2.216 ± 0.109	2.929 ± 0.314
	40	1.753 ± 0.234	2.309 ± 0.134
	50	1.390 ± 0.105	1.962 ± 0.178
Ni	5	1.524 ± 0.065	2.60 ± 0.119
	10	1.786 ± 0.176	2.852 ± 0.178
	20	1.800 ± 0.15	3.115 ± 0.085
	40	1.453 ± 0.138	2.219 ± 0.219
	50	1.208 ± 0.027	2.005 ± 0.1
Pb	5	1.550 ± 0.127	2.508 ± 0.127
	10	1.792 ± 0.158	2.705 ± 0.144
	20	1.850 ± 0.061	2.923 ± 0.143
	40	1.453 ± 0.052	2.5 ± 0.14
	50	1.181 ± 0.095	2.012 ± 0.123
Zn	5	1.712 ± 0.112	2.523 ± 0.121
	10	1.928 ± 0.118	2.824 ± 0.145
	20	2.317 ± 0.212	3.072 ± 0.138
	40	2.507 ± 0.097	3.281 ± 0.199
	50	2.601 ± 0.151	3.402 ± 0.122

† Values are averages of three replicates ± S.D.

3.7. Heavy metal uptake by plant tissue

The mean uptake of all five metals by sunflower plants increased as the concentration of these metals in the soil- vermin-composting media increased (Table 5). In this plant the heavy metals, Cu, Cd , Ni and Zn were taken up by shoot and root both; where Pb concentration was found to be higher in roots. The heavy metals were untaken by the sunflower plants in the following order: Zn> Cu>Cd>Ni>Pb. Several studies have demonstrated that the metal concentration in the plant tissue is a function of the heavy metals content in the growing environment (Cui et al., 2004; Grifferty and Barrington, 2000). Uptake is largely influenced by the availability of metals, which is in turn determined by both external (soil-associated) and internal (plant associated) factors. The roots preferentially explored metals in the contaminated area. The exploration of polluted soil inclusions by the roots was associated with higher extraction of metals. Many plant species or ecotypes associated with heavy-metal enriched soils accumulate metals in the shoots. These plants can be used to clean up metal-contaminated sites by extracting metals from soil and concentrating them in aboveground biomass (Peng et al., 2006).

The metal-enriched biomass can be harvested using standard agricultural methods and smelted to recover the metals. Plant growing in metal contaminated environment can accumulate toxic metal ions and efficiently compartmentalize these into various plant parts. Several studies indicated that the partitioning of heavy metals at the whole plant level could broadly be divided into three categories. For instance, Chaney and Giordano classified Mn, Zn, Cd, B, Mo and Se as elements, which were readily translocated to the plant shoots; Ni, Co and Cu, were intermediate, and Cr, Pb and Hg were translocated to the lowest extent (Alloway, 1995).

Some species, such as cabbage (*Brassica oleracea L.*), lettuce (*Latuca sativa L.*) and tobacco (*Nicotiana tabacum L.*), accumulate high levels of Cd in leaves rather than in roots and increases or decreases the bioavailability of metal ions. In plants Cd damages the light harvesting complex II and photosystems II, and I, which are active in photosynthesis. Total chlorophyll content is decreased by Cd treatment, and nonphotochemical quenching is increased in *Brassica napus*. Probably Cd also interferes with movement of K^+, Ca^{2+} and abscisic acid in guard cells, while inhibiting stomal opening (Shaw, 1995). The root of Indian mustard are found to be effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn, and sunflower can remove Pb, U, Cs,-137 and Sr-90 from hydroponic solutions (Lone et al., 2008).

Tang et al. (2003) reported the increase in uptake of copper by Indian mustard and sunflower plant. Nehnevajova et al., (2005) investigated that the highest metal concentration was found in leaves (shoot) of commercial cultivars of sunflower plants grown on metals-contaminated soil.

Among the cultivated crops rape and sunflower revealed higher cadmium concentrations in their shoots than in the roots.

Significantly more copper was found in grains and straw of oat treated with vermin-compost than in the objects where mineral fertilizers were used (Gondek and Filipek-Mazur, 2003). UNEP (2002) mentioned the application of sunflower plantation for phytoremediation in Silesia, Poland where heavy metals were directly applied on the top soil by dispenser designed at the Institute for Ecology of Industrial Areas-Katowice, Poland.

Studies done in sunflower plant showed that the maximum concentration of Pb was found in root and leaf (shoot) was the major organ of Zn accumulation (Herrero et al., 2003). At a Department of Energy site in Ashtabula, Ohio, sunflower plants were used to help clear uranium from surface water and groundwater.

Table 5. Metal concentration in roots and shoots and uptake (compared to control treatmet).†

Metal	*Dose (ppm)*	*Roots Metal uptake (ppm)*	*Shoots Metal uptake (ppm)*
Cd	5	0.619 ± 0.019	1.105 ± 0.057
	10	1.602 ± 0.087	2.349 ± 0.174
	20	2.105 ± 0.057	4.816 ± 0.108
	40	4.168 ± 0.040	9.021 ± 0.162
	50	5.211 ± 0.129	11.527 ± 0.131
Cu	5	0.908 ± 0.048	1.613 ± 0.087
	10	1.327 ± 0.045	2.719 ± 0.039
	20	2.685 ± 0.054	5.276 ± 0.074
	40	5.029 ± 0.073	10.781 ± 0.088
	50	6.078 ± 0.074	13.162 ± 0.153
Ni	5	0.517 ± 0.048	1.318 ± 0.042
	10	1.275 ± 0.074	2.152 ± 0.024
	20	2.316 ± 0.045	4.701 ± 0.074
	40	3.961 ± 0.257	8.138 ± 0.125
	50	4.673 ± 0.092	12.152 ± 0.145
Pb	5	0.724± 0.031	0.287 ± 0.027
	10	1.001 ± 0.097	0.602± 0.049
	20	2.862 ± 0.061	0.975 ± 0.074
	40	4.784 ± 0.171	1.364 ± 0.076
	50	6.052 ± 0.119	1.961± 0.064
Zn	5	1.253 ± 0.045	2.972 ± 0.07
	10	2.014± 0.084	5.162 ± 0.099
	20	5.132 ± 0.063	9.084 ± 0.083
	40	8.019 ± 0.083	16.327 ± 0.212
	50	9.205 ± 0.166	20.815 ± 0.314

† Values are averages of three replicates ± S.D.

The same technique was used in a pond within a kilometer of the Chernobyl nuclear generating plant in the Ukraine where an explosion and fire in 1986 caused the world's most serious nuclear accident spreading radioactive contamination across much of Europe (Wikimedia Foundation, Inc 2006).

The trial with uranium-contaminated water was carried out at U concentrations of 100-400ppb. Roots of a selected sunflower cultivar (*Helianthus annuus L.*) caused the uranium solution concentration to decline by 95% within the first 24 hours. A similar approach was used to evaluate the use of plants to treat water contaminated with cesium and strontium in the Ukraine. A natural pond with a surface area of about 75 square meters (m^2) near the village of Yanov was selected as a site for a rhizofiltration field trial. Radionuclide concentrations in the basin were approximately 80 Bq/L for cesium-137 and 1200Bq/L for strontium-90 as a result of the 1986 release by the nearby Chernobyl nuclear reactor. Radionuclide uptakes studied were carried out directly in the contaminated pond. After 4 to 8 weeks of hydroponic growth in the pond, sunflower plants were harvested dried, and the radionuclide concentration measured. The plant tissue concentrations of cesium-137 reached 6.4 x 10 5 Bq per kilogram (dry mass) and 2.5 x 10 6 Bq of strontium-90 per kilogram dry mass (Ensley et al., 1997). The phytoavailable lead is usually very low due to its strong association with organic matter, Fe-Mn oxides, clays and precipitation as carbonates, hydroxides, and phosphates (Shen et al., 2002). The uptake of Pb by 5.6 +1.3 mg in shoot and 61.6 +3.3 mg in root of sunflower plant has been investigated by Nandkumar et al. (1995). Boonyapookana et al. (2005) showed that 80- 87% of the total Pb uptake was localized in plant roots of *H. annuus* with only 13-20% translocated to the aboveground parts (shoot) after the fourth week of studies. He also reported that very low concentration of the Pb is translocated to aerial plant tissues. The X-ray map of root tissue of sunflower revealed the spots of Pb, forming patterns of concentric circles inside the stele, more precisely in the central region of vascular bundles. These spots were also visible on the surface of root epidermis, in the parenchyma cells of the cortical region and outside the endodermis. When Pb enters the plant root, it immediately comes in contact with high phosphate concentrations, relatively high pH, and high carbonate-bicarbonate concentrations in the intercellular spaces. Pb precipitates out of solution in the form of phosphates or carbonates that can be seen in electron micrographs of roots. Plant roots contain inclusion bodies of these forms of Pb in the tissue, reduce Pb translocation in plants.

Plants use photosynthetic energy to extract ions from the soil and concentrate them in their biomass, according to nutritional requirements (Kramer and Chardonnens, 2001). The essential elements (Cu and Zn) are required in low concentrations and hence are known as trace elements or micronutrients, whereas nonessential elements (Cd, Ni, and Pb) are phytotoxic (Gerard et al., 2000). Zn is relatively mobile in soils and is the most abundant metal in root and shoot of contaminated plants as it is in soils. This metal is necessary as a minor nutrient and it is known that plants have special zinc transporters to absorb this metal (Zhu et al., 1999). However, an excessive accumulation of this element in living tissues leads to toxicity symptoms.

In this experiment, shoot is the major organ of heavy metals accumulation (except Pb). Cu is the second most abundant element in contaminated plants and in soils. When the contamination increased, a significantly higher content of copper was found in shoots than roots. Cadmium also is considered to be mobile in soils but is present in much smaller concentrations than Zn (Zhu et al., 1999). Higher Cd concentrations are generally observed in plants grown on soil subjected to the metal-processing industry, sludge application, and long-term fertilization, with up to 70 mg Cd kg^{-1} DM in lettuce (Gerard et al., 2000). Moreover, many studies have demonstrated that Cd taken up by plants accumulates at higher concentrations in the roots than in the leaves (Boominathan and Doran, 2003). Alloway (1995) reported that *Alyssum* species which are naturally adapted to serpentine soils can accumulate over 2% Ni. The uptake by some plants has been confirmed for Cd (up to 0.2% Cd in shoot dry biomass), Ni (up to 3.8% Ni in shoot dry biomass) and Zn (up to 4% Zn in shoot dry biomass) by Kramer and Chardonnens (2001). The application of peat and manure in contaminated soil increased Cu, Zn, and Ni accumulation by wheat (Schmidt, 2003).

Organic matter in soil could effectively increase the activity of metals in soil and improve metal mobility and distribution in soil. The application of natural fertilizer (compost and vermin-compost) in soils has helped in increase in metal mobility through the formation of soluble metal-organic complexes (Yang et al., 2005). In addition, exudation of organic compounds by plant roots, such as organic acids, influence ion solubility and uptake (Klassen et al., 2000) through their effects on microbial activity, rhizosphere physical properties, and root-growth dynamics.(Yang et al., 2005).

Plant root exudation is of particular importance due to their metal chelating/complex forming properties for the mobilization of mineral nutrients and heavy metals (Jauert et al., 2002).

4. Conclusions

This research work deals with phytoremediation of heavy metals by sunflower in the soil - Vermin-compost media. The phytoremediation techniques for the heavy metal management proves to be very effective as its cost is approximately one tenth that of conventional soil cleansing procedures, and in some cases, the plant material can be further utilized to recoup the cost of the operation or even turn a profit. Another advantage of phytoremediation is that it leaves the soil fertile and has less adverse environmental effects as compared to conventional procedures.

The research finding shown that the low doses of heavy metals applied stimulated the root and shoot elongation of sunflower plants. At higher concentrations i.e. 40 and 50 ppm of Cd, Cu, Ni and Pb significantly reduced the ability to germinate. However, the plants were able to germinate and grow efficiently at any Zn concentration evaluated in this study. The study shows that heavy metals were efficiently up taken at all concentrations using high biomass producing plant *Helianthus annuus* grown in vermicompost media and the uptake was increased along the increasing concentrations in soil.

The sunflower seed and sunflower oil may be used for technical lubricants or energy production and metal-enriched biomass can be harvested using standard agricultural methods and smelted to recover the metals.

The present technology will help to remediate the higher concentrations of metals by the application of vermin-compost as a natural fertilizer in soil. This technology will be applicable at the site to remediate the heavy metals. Thus, an increase in the plant resistance to heavy metal toxicity (Zn, Cu, Ni, Pb and Cd) seems possible by means of addition of nutrient (vermincompost) supply.

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