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Phytoremediation: The Application of Vermicompost to Remove Heavy Metals by Green Plants (Alfalfa, Sunflower and Sorghum)

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ABSTRACT

Vermicompost – a natural fertilizer – was developed by vermiculture biotechnology using market vegetable wastes. The collected vegetable waste was decomposed for a period of two weeks following mesophilic, thermophlic and cooling down stages which then subjected to subsequent vermicomposting for a period of two months. The developed vermicompost was characterized for its chemical and nutrient status. It was found to have high concentrations of the nutrient elements (Zn, Cu, Mg, Fe and Mn) and thus was used as an organic amendment in the soil (at the 3: 1 ratio) for enhancing the uptake capabilities of plant species in the phytoremediation studies. Glasshouse pot culture experiments were then conducted to investigate the phytoremediation potential of three plants- alfalfa, sunflower and fibrous root grass (sorghum) for the removal of heavy metals from the contaminated soil-vermicompost media. The soil-vermicompost media used in pot experiment were dosed separately at varying concentrations (0 to 50 ppm) of heavy metals; Cd, Ni, Cu, Pb and Zn to study the effect of heavy metals on seed germination and root and shoot growth of the plants. The uptake of metals by the three plants was also assessed. The results indicated that seed germination and root and shoot growth were significantly affected by these metals at higher concentrations ($P<0.05$). Zinc being an essential trace nutrient, it was found to be the only metal that caused significantly positive effect on plants at concentrations 20 to 50 ppm ($P<0.05$). All the three plants were observed to be significantly effective in uptake of heavy metals at all the evaluated concentrations. However, the findings of these phytoremediation studies demonstrate that accumulation potential of sunflower was found be greater than that of sorghum and alfalfa in the soil-vermicompost media.

Keywords: contamination, earthworm, phytotoxicity, nutrient uptake, vegetable waste

INTRODUCTION

The rapid industrialization and urbanization have dramatically increased the generation of wastes and new types of pollutants. Activities such as mining, smelting of metal ores, uncontrolled disposal of waste, application of insecticides, fertilizers, sewage sludge to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leachate contributing towards contamination of our ecosystem (WHO 2008; Memon and Peter 2009). Though metals are natural components in soil, contamination of heavy metals represents one of the most pressing threats to water and soil resources as well as human health (Kabata-Pendias and Pendias 2001; Ferré-Huguet *et al*. 2008). The most common heavy metal contaminants are: Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Nickel (Ni) and Zinc (Zn) (USEPA 1997). Exponential increase in the use of heavy metals in industrial processes and products, human exposure to heavy metals has risen dramatically in the last 50 years. Worldwide, the contamination of soils by metals has been recognized as an important environmental issue. Remediation of polluted soils has to be considered when potentially toxic elements or substances in soils impose adverse effects on biodiversity or human health. Although traditional technologies for cleaning contaminated soils and waters have proven to be efficient, they are usually expensive, labor intensive and in the case of soil, they produce severe disturbance. More recently, the use of plants in metal extraction (phytoremediation) has emerged as a promising alternative in the removal of heavy metal contamination from soil and water (Bennett *et al*. 2003).

Phytoremediation is a recent technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long term applicability (Su and Wong 2004; Boonyapookana *et al.* 2005). 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 and rhizofiltration (Schnoor 1997). Phytoremediation is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. The importance of employing effective agronomic practices has been discussed by Chaney *et al*. (2000). Biological processes such as composting followed by vermicomposting to convert vegetable waste (as valuable nutrient source) in agriculturally useful organic fertilizer would be of great benefit (Jadia and Fulekar 2008b).

In India, about 320 million tones of agricultural wastes are generated annually of which vegetable waste alone is in major proportion (Suthar *et al*. 2005). The waste from the vegetable market is collected and dumped into the municipal land fills, causing a nuisance because of high biodegradability (Bouallagui *et al*. 2004). These results in loss of potentially valuable materials that can be processed as fertilizer, fuel and fodder (Baffi *et al*. 2005). The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact (Coker 2006; Paraskeva and Diamadopoulos 2006). A possible way to utilize this waste is proposed to be vermicomposting bio-

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technology (Benifez *et al*. 1999; Mills 2006). 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. Therefore, natural fertilizer or vermicompost is found far more superior in respect of nutrient value, colour, flavor and quality.

The composting of vegetable waste followed by vermicomposting with earthworms (*Eisenia foetida*) develops it into a natural fertilizer (Maharashtra Nature Park Society 2003). The vermicompost contains high nutrient value, increases fertility of soil and maintains soil health (Suthar *et al*. 2005). Leachate from vermicomposting contains large amounts of plant nutrients and stimulated plant development and can be used as liquid fertilizer (Gutiérrez-Miceli *et al*. 2008).

Application of compost and vermicompost in contaminated soil is reported to improve soil fertility and physical properties as well as helps in successful approach to phytoremediation (Zheljazkov and Warman 2004; Jadia and Fulekar 2008a). Several researchers have demonstrated that earthworm castings (excretory pellets) have excellent aeration, porosity, structure, drainage, and moisture-holding capacity. The vermicompost is a rich source of beneficial microorganisms and nutrients (Paul 2000) and used as a soil conditioner or fertilizer (Elcock and Martnes 1995; Hattenschwile and Gaser 2005). 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 vermicompost developed from vegetable waste by vermiculture biotechnology with soil would provide natural environment for phytoremediation (Elcock and Martens 1995; Jadia and Fulekar 2008c).

In the work presented we address the application of vermicompost developed from vegetable waste in the soil, contaminated with heavy metals for phytoremediation studies using three green plants. Alfalfa, sunflower and sorghum were the chosen deep rooted plants used in the experiments which have great potential for phytoremediation of the heavy metals. The objectives of this study is to determine the effects of heavy metals on seed germination, plant growth and examine their uptake by alfalfa, sunflower and grass with fibrous roots (sorghum) in soil- vermicompost media.

MATERIALS AND METHODS

Soil sampling and characterization

Soil was collected in random sample plots 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 in the laboratory. The soil was screened through 2 mm stainless steel sieve, and stored in a plastic bag at room temperature (28-30°C) until use. Concentrations of Pb, Zn, Cu, Ni, and Cd were determined by atomic absorption spectrophotometer (APHA 1998) while the physicochemical parameters were measured by standard methods (**Table 1**). Soil texture was determined by the Bouyoucos hydrometer method and the moisture content calculated by the weight difference before and after drying to a constant weight at 105°C. The pH and electrical conductivity (EC) were measured after 20 min of vigorous mixing of samples in 1: 2.5 (solid:deionized water), 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 using the Walkley–Black method (Jackson 1973).

Table 1 Physicochemical properties of experimental soil[†].

Soil parameters	
Clay %	25.9 ± 1.8
Silt %	21.7 ± 2.5
Sand %	50.4 ± 2.8
pH	7.2 ± 0.1
Organic matter %	0.80 ± 0.045
Nitrogen %	0.05 ± 0.01
CEC 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 metal ppm	
Cu	3.6 ± 0.5
Cd, Ni, Pb	ND
Zn	12 ± 1.5

[†]: Values are averages of three replicates ± S.D., CEC: Cation exchange capacity, EC: Electrical conductivity, WHC: Water Holding Capacity, ND: Not detected

Vermicompost production

The vermicompost was produced from chopped pieces (2-5 mm size) of vegetable waste (cabbage, French bean, cauliflower, okra, spinach, carrot and radish) collected from local market. Vermicomposting was carried out in rectangular perforated (sides and bottom) plastic boxes of dimensions [2(l) × 1 (b) × 1 (h)] ft, with working volume of 15-kgs. Each box was equipped with aeration and stirrer system for turning of composting materials. The turning of composting materials was done twice a day for a period of 15 days. The moisture content, temperature and pH of composting materials were maintained at 55 to 65%, 30 to 33°C and 6.8 to 7.2, respectively. The bulking agents such as sawdust, tree bark, straw, and dry leaves were added to maintain desirable moisture content in the composting process. The temperature was cooled down after the thermophilic conditions and allowing to stabilize at 30-33°C by turning of composting materials. The pH value of the composting materials was maintained by adding caustic lime to the raw materials.

Exotic varieties of earthworms (*Eisenia foetida*) were obtained from Maharashtra Nature Park, Mahim Dharavi (Mumbai) and about ½ Kg (250 earthworms) was used in vermicomposting. The physicochemical parameters were measured during vermicomposting as described above for soil analysis. After 2.5 months, vermicompost was collected, air dried, sieved (2-mm) and a portion of it was taken for nutrient analysis in order to determine its suitability as biofertilizer. The nutrients in dried vermicompost sample were digested with concentrated nitric acid and 30% hydrogen peroxide and then quantified by an atomic absorption spectrophotometer [AAS, Perkin Elmer] (APHA 1998). Nutrient and trace elements content in the vermicompost are presented in **Table 2**.

Phytoremediation experiments

Pot culture experiments were conducted in the greenhouse to study the effect of heavy metals (Cd, Cu, Ni, Pb and Zn) on seed germination, root/shoot growth and phytoremediation (metal uptake) by alfalfa, sunflower and grass with fibrous roots (sorghum). The growth medium in the pots consisted of alluvial soil and vermicompost (3: 1). The growth medium was 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 $(NO_3)_2.6H_2O$. Each heavy metal was applied at 0, 5, 10, 20, 40 and 50 ppm levels. Seeds of alfalfa (*Medicago sativa*), fibrous root grass (*Sorghum bicolor*), and sunflower (*Helianthus annuus*) were obtained from local seed supplier Ratanshi Agro-Hortitech (Byculla, Mumbai) and immersed in 3% (v/v) formaldehyde solution for 5 min, followed by washing several times with distilled water to remove fungal contaminants.

To determine the effect of heavy metals on seed germination, 30 uniform seeds of each plant were placed in each Petri dish containing soil-vermicompost media amended with heavy metals at various concentrations (0, 5, 10, 20, 40 and 50 ppm). The Petri dish lids were left partially open and kept in the dark to observe germination. The seeds were considered germinated when the radicles emerged. Ten plants of each species were grown in each small plastic pots containing 250 g soil-vermicompost media amended with heavy metals at various concentrations (0 to 50 ppm) for studying effect of metals on shoot and root growth. Similarly, in a separate set ten plants of each species were grown in each 2 kg capacity plastic pots for phytoremediation study. Soil moisture content was adjusted regularly using deionized water to about 60% of water-holding capacity. To prevent loss of nutrients and trace elements out of the pots, plastic trays were placed under each pot and the leachates collected were put back in the respective pots. Each treatment was replicated thrice. The seeds were set under 12/12 hrs light/dark cycle and temperatures of 30°C during the day and 27°C during the night, at 75% average relative humidity. The seedlings were removed after four days for determination of germination rate and shoot/root length was evaluated after 2 weeks. For the phytoremediation study plants were harvested after 10 weeks and each plant was separated into roots and shoots. The plant samples were washed with distilled water and dried in an oven at 70°C for 3 days, and weighed to determine the dry weight. The samples were stored in brown paper bags until digestion with concentrated nitric acid and 30% hydrogen peroxide and determination of heavy metal content using an atomic absorption spectrophotometer [AAS, Perkin Elmer] (APHA 1998).

Statistics

Each treatment for percentage seed germination, plant root/ shoot growth and uptake consisted of three replicates for statistical purpose. The data presented for each treatment in this study is represented as mean of samples with standard deviation (X ± S.D.) and the student t-test was also performed to test the significance of differences between the control and treated plants (Mahajan 1997).

RESULTS AND DISCUSSION

The present research work has been carried out to study the uptake of heavy metals by the green plants amended in soil-vermicompost media. The results obtained by the study are presented next.

Soil analysis

The sandy loam texture affected soil properties including its water supplying power, rate of water intake, aeration, fertility and ease of tillage (**Table 1**). The soil pH of 7.2 is within the recommended value for proper growth and efficient uptake of nutrients and compounds from soil. Macronutrients including metals were also present in substantial amount but there was no history of heavy metal (Cd, Ni, and Pb) contamination.

Vermicompost analysis

Upon characterization, the vermicompost was found to have high concentration of nutrients such as Zn, Cu, Mg, Fe, and Mn (**Table 2**). The vermicompost was thus ascertained to be a rich source of beneficial microorganisms and nutrients, as reported by Paul (2000) and can also be used as a soil conditioner (Hattenschwile and Gaser 2005). Ma *et al.* (2002, 2003) found that species of *Pheretima* increased the mobility of metals in Pb/Zn mine tailing diluted with uncontaminated soil. They suggested that earthworms may benefit attempts to use plants for phytoextraction by increasing the amount of metal in the soil available for plant uptake. The presence of *Lumbricus terrestris* in microcosms increased the concentration of Cd, Cu, and Zn into roots and shoots (and of Pb and Fe into roots) of *Lolium perenne* seedlings (Rida 1996). An experiment was conducted by Azarmi *et al.* (2008) revealed that addition of vermicompost at rate of 15 t ha^{-1} significantly (at $P<0.05$) increased growth, elemental content of tomato plant (*Lycopersicum esculentum* var. Super Beta) and yield compared to control treatment. Some evidence indicates that earthworms increase metal bioavailability in relatively low-level metal-contaminated soils with higher organic matter contents (Cheng and Wong 2002).

Table 2 Chemical and nutrient status of vermicompost. †

Parameters	
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 /100 g	54.68 ± 9.44
Magnesium mg/100 g	832.48 ± 22.48
Iron mg/100 g	746.26 ± 23.39
Zinc mg/100 g	16.19 ± 0.55
Manganese mg/100 g	53.86 ± 2.84
Copper mg/100 g	5.16 ± 0.36

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

Table 3 Comparison of seed germination (%) of three green plants: alfalfa, sunflower and sorghum in heavy metals (Cd, Cu, Ni, Pb and Zn) contaminated soil†.

Metal	Dose (ppm)	Alfalfa	Sunflower	Sorghum
Cd	0	80 ± 4	70 ± 4	75 ± 3
	5	77 ± 5	65 ± 4	73 ± 4
	10	70 ± 4	60 ± 3	68 ± 2.5
	20	65 ± 5	53± 3	61 ± 3
	40	57 ± 7	46 ± 6	50 ± 3
	50	49 ± 7	40 ± 5	43 ± 4.6
Cu	5	83 ± 7	75 ± 5	77 ± 4.7
	10	85 ± 6	63 ± 4	80 ± 6
	20	72 ± 7	60 ± 7	70 ± 2.4
	40	60 ± 4	55 ± 3.05	66 ± 4
	50	58 ± 6	50 ± 6	60 ± 3
Ni	5	79 ± 7	66 ± 4	74 ± 3
	10	75 ± 4	63 ± 4	70 ± 3
	20	70 ± 8	56 ± 3	63 ± 5
	40	64 ± 5	48 ± 5	56 ± 3
	50	60 ± 7	43 ± 2	50 ± 4
Pb	5	79 ± 8	70 ± 7	72 ± 4
	10	77 ± 5	57 ± 5.50	69 ± 3
	20	74 ± 6	56 ± 3.05	65 ± 4
	40	66 ± 5	50 ± 4	60 ± 4
	50	63 ± 3	46 ± 4	58 ± 6
Zn	5	81 ± 5	74 ± 5.56	76 ± 1
	10	83 ± 6	76 ± 7	80 ± 4
	20	88 ± 5	84 ± 5	86 ± 3
	40	91 ± 5	92 ± 7.37	92 ± 5
	50	95 ± 3	94 ± 4	97 ± 2

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

Effect of heavy metals on seed germination

The data in **Table 3** clearly shows that percentage of seed germination decreased as metals concentration increased in the soil-vermicompost media. The percentage seed germination of control treatment of alfalfa, sunflower and sorghum were 80, 70 and 75%, respectively (**Table 3**). The seed germination of the experimental plants significantly dropped at the higher doses (40 and 50 ppm) of metals ($P<0.05$). The resulting order of toxicity for metals on seed germination was Cd > Cu > Ni > Pb > Zn. Delayed germination was also observed in all cases at higher i.e. 40 and 50 ppm concentrations. However, in the same study Zn being the only metal which did not reduce the seed germination of alfalfa, sunflower and sorghum but significantly increase the rate of germination at concentrations 20 to 50 ppm ($P<0.05$). The experiment conducted by Peralta-Videa *et al.* (2004) showed that the susceptibility of living alfalfa plants to Cd, Cu and Zn was correlated to the age of the plants. Further their work also elaborated that after four days of germination; Cr, Cd, Ni, except Zn, had lethal effects on the alfalfa seedlings. The heavy metals (Cd and Ni) at higher concentration decreased the seed germination and growth of sunflower plant (Khan and Moheman 2006). Seed germination and

Table 4 Comparison of root length of three green plants: alfalfa, sunflower and sorghum in heavy metals (Cd, Cu, Ni, Pb and Zn) contaminated soil[†].

Metal	Dose (ppm)	Alfalfa	Sunflower	Sorghum
Cd	0	4.9 ± 0.8	9.1 ± 0.50	6.50 ± 0.10
	5	5.5 ± 0.8	9.7 ± 0.6	5.27 ± 0.29
	10	4.5 ± 0.9	10.5 ± 0.71	5.00 ± 0.70
	20	3.9 ± 0.7	8.5 ± 0.31	4.24 ± 0.74
	40	3.4 ± 0.3	7.1 ± 0.43	3.15 ± 0.35
	50	2.9 ± 0.4	5.8 ± 0.88	2.10 ± 0.25
Cu	5	5.1 ± 0.55	10 ± 0.86	6.82 ± 0.71
	10	5.6 ± 0.48	11.3 ± 0.91	7.13 ± 0.67
	20	6.5 ± 0.34	13.8 ± 0.61	4.44 ± 0.34
	40	4.1 ± 0.54	8.7 ± 0.2	3.63 ± 0.53
	50	3.8 ± 0.82	8.3 ± 0.5	3.05 ± 0.75
Ni	5	5.2 ± 0.52	9.8 ± 0.68	6.70 ± 0.60
	10	5.4 ± 0.56	10.7 ± 1.58	6.90 ± 0.70
	20	4.8 ± 0.64	12.3 ± 1.5	5.10 ± 0.80
	40	4.5 ± 0.55	8.4 ± 0.5	4.20 ± 0.50
	50	4.1 ± 0.4	7.2 ± 0.62	3.10 ± 0.80
Pb	5	5 ± 0.4	10.64 ± 1.54	6.60 ± 1.15
	10	5.3 ± 0.55	11.45 + 1.15	6.80 ± 0.65
	20	4.7 ± 0.59	13.2 ± 1.4	6.10 ± 0.60
	40	4.3 ± 0.25	8.6 ± 0.5	4.10 ± 0.55
	50	4 ± 0.38	7.9 ± 1.2	3.80 ± 0.65
Zn	5	5.1 ± 0.73	11.9 ± 1.69	7.00 ± 0.80
	10	5.4 + 0.27	14.3 ± 1.52	7.80 ± 0.63
	20	6.1 ± 0.28	15.1 ± 1.3	9.40 ± 0.55
	40	6.8 ± 0.54	15.8 ± 0.81	10.9 ± 0.80
	50	7.5 ± 0.67	17.3 ± 1.47	12.2 ± 1.08

[†]: Values are averages of three replicates ± S.D.

Table 5 Comparison of shoot length of three green plants: alfalfa, sunflower and sorghum in heavy metals (Cd, Cu, Ni, Pb and Zn) contaminated soil[†].

Metal	Dose (ppm)	Alfalfa	Sunflower	Sorghum
Cd	0	5.5 ± 0.6	7.5 ± 0.7	13.18 ± 1.86
	5	5.3 ± 0.6	18.1 ± 0.8	14.40 ± 1.70
	10	4.7 ± 0.5	20.3 ± 0.82	12.81 ± 1.69
	20	3.8 ± 0.4	16.2 ± 0.27	11.20 ± 1.01
	40	4.1 ± 0.5	12.6 ± 0.65	9.100 ± 0.85
	50	3.6 ± 0.5	0.01 ± 0.74	7.200 ± 1.35
Cu	5	5.7 ± 0.38	17.8 ± 1.88	14.92 ± 1.74
	10	6.7 ± 0.77	18.3 ± 0.76	16.42 ± 1.25
	20	7.8 ± 0.89	19.5 ± 1.38	14.75 ± 0.75
	40	4.7 ± 0.44	16.7 ± 1.18	12.12 ± 1.62
	50	4 ± 0.79	14.4 ± 1.28	9.930 ± 1.13
Ni	5	5.7 ± 0.76	17.2 ± 1.39	14.90 ± 1.65
	10	5.2 ± 0.9	19.2 ± 0.9	14.48 ± 1.38
	20	5 ± 0.8	20.2 ± 3.1	13.70 ± 2.05
	40	4.7 ± 0.54	17.3 ± 1.7	10.20 ± 1.70
	50	4.5 ± 0.5	14.3 ± 1.3	9.500 ± 1.55
Pb	5	5.9 ± 0.97	18.3 ± 1.5	16.70 ± 1.75
	10	5.3 ± 0.27	19.4 ± 1.4	15.60 ± 1.25
	20	5 ± 0.64	20 ± 1.5	15.20 ± 1.23
	40	4.9 ± 0.64	14.5 ± 1.19	10.60 ± 1.45
	50	4.7 ± 0.58	16.2 ± 1.35	9.200 ± 1.65
Zn	5	5.6 ± 0.48	18.5 ± 2.29	16.40 ± 1.22
	10	6.8 ± 0.82	19.7 ± 1.4	16.90 ± 1.80
	20	7.2 ± 0.68	21.6 ± 2.04	17.44 ± 1.29
	40	7.7 ± 0.78	22.8 ± 2..25	19.80 ± 1.85
	50	8.2 ± 0.55	24.7 ± 2.2	21.00 ± 1.40

[†]: Values are averages of three replicates ± S.D.

growth of sorghum seedlings was found to be reduced at 40 and 50 ppm of heavy metals (Ganesh *et al*. 2006). Farooqi *et al.* (2009) investigated that seed germination of *Albizia lebbeck* L., was highly decreased with the treatment of Pb and Cd at 10, 30, 50, 70 and 90 μmol/L as compared to control treatment.

Effect of heavy metals on root growth

An increase in the heavy metal concentration (from 20 to 50 ppm) in the soil- vermicompost media caused significant decrease in root length with stunt growth of roots ($P<0.05$) (**Table 4**). There was reduction in the formation of secondary roots and number of root hairs by Cd metal at 40 and 50 ppm in all the three plants. The root length of control treatment of alfalfa, sunflower and sorghum were 4.9, 9.1 and 6.5, respectively as shown in **Table 4**. All the Zn concentrations (20 to 50 ppm) significantly increased the root length than the control root length of the treated plants. Nandakumar *et al*. (1995) demonstrated that root growth of Pb treated sunflower plants was retarded compared to the controls. Presence of heavy metals at higher concentrations in the soil- vermicompost medium decreased plant root length and caused stunted growth of sorghum roots (Jadia and Fulekar 2008a). Farooqi *et al.* (2009) showed that the root, shoot and dry biomass of *Albizia lebbeck* L., were highly decreased with the treatment of Pb and Cd at 10, 30, 50, 70 and 90 μmol/L as compared to control treatment.

Effect of heavy metals on shoot growth

The effects of heavy metals on the shoot growth are different from their effects on root growth. The results showed that, after two weeks of growth, the shoot elongations of plants at higher concentration (50 ppm) of all the four metals that is; Cd, Ni, Pb and Cu were significantly less than that observed for the control plants ($P<0.05$) (**Table 5**). The shoot length of control treatment of alfalfa, sunflower and sorghum were 5.5, 17.5 and 13.18 respectively as shown in **Table 5**. The plants exposed to metals at 5 to 10 ppm grew taller than the control plants, suggesting that plants (alfalfa, sunflower and sorghum) seedlings were able to sustain the adverse effects of the treatments. All three plant species; alfalfa, sunflower and sorghum showed visible symptoms of phytotoxicity including chlorosis and necrosis after exposure to the high Cd treatment. On the other hand, Zn concentrations (20-50 ppm) significantly increased the shoot length than the control root length of alfalfa, sunflower and sorghum. Statistically significant differences ($P<0.05$) between the shoot length of the control treatment plants and the length of plants of alfalfa grown in the presence of the heavy metal mixture was reported by Peralta-Videa *et al*. (2002). Stunted growth and visual toxicity symptom of cadmium (Cd 180) in shoot of sunflower plant was demonstrated by Singh (2006). Jadia and Fulekar (2008a) showed that low concentrations of Cd, Cu, Ni, and Pb have micronutrient-like effects on the sorghum plants.

Heavy metal uptake by plant tissue (roots and shoots)

Metal concentrations in plant tissues also differed among the three plant species grown on the same soils, indicating their different capacities for metal uptake. The result showed that mean uptake of all five metals by plants significantly increased as the concentration of these metals in the soil-vermicompost media increased ($P<0.05$) as compared with the control plants which were grown without metal amendment (0 ppm) (**Table 6A-C**). The heavy metals were taken up by the alfalfa, sunflower and sorghum plants in the following order: Zn > Cu > Cd > Ni > Pb (**Table 6A-C**). In alfalfa the heavy metals (Cd, Ni and Pb) were taken up by higher concentrations in roots than shoots, whereas Cu and Zn contents were found higher in shoots than roots. On the contrary, in case of sunflower plant the heavy metals, Cd, Cu, Ni and Zn were taken up by higher concentrations in shoots than roots; whereas Pb concentration was found to be higher in roots. The uptake of five different heavy metals by fibrous root grass (sorghum) was observed majorly in the roots.

Plants have developed a range of mechanisms to obtain metals from the soil solution and transport these metals

Table 6A Phytoremediation (uptake) of heavy metals (Cd, Cu, Ni, Pb and Zn) by alfalfa in contaminated soil. [†]

Metal	Dose (ppm)	Alfalfa	
		Roots metal uptake (ppm)	Shoots metal uptake (ppm)
Cd	5	0.732 ± 0.05	0.152 ± 0.01
	10	0.817 ± 0.03	0.198 ± 0.01
	20	2.032 ± 0.09	0.652 ± 0.03
	40	3.816 ± 0.11	1.557 ± 0.05
	50	5.928 ± 0.13	2.715 ± 0.07
Cu	5	1.302 ± 0.08	2.710 ± 0.17
	10	1.850 ± 0.19	3.620 ± 0.02
	20	2.380 ± 0.19	5.460 ± 0.25
	40	3.690 ± 0.18	7.800 ± 0.2
	50	5.380 ± 0.12	9.750 ± 0.24
Ni	5	1.108 ± 0.01	0.473 ± 0.01
	10	1.595 ± 0.09	0.708 ± 0.01
	20	3.625 ± 0.10	0.834 ± 0.01
	40	5.216 ± 0.09	1.102 ± 0.09
	50	5.905 ± 0.03	1.575 ± 0.07
Pb	5	0.417 ± 0.03	0.098 ± 0.01
	10	0.789 ± 0.02	0.175 + 0.02
	20	1.780 ± 0.08	0.334 ± 0.02
	40	2.701 ± 0.08	0.452 ± 0.02
	50	3.890 ± 0.18	0.614 ± 0.07
Zn	5	1.500 ± 0.03	3.500 ± 0.05
	10	2.170 ± 0.09	4.010 ± 0.17
	20	2.950 ± 0.15	8.230 ± 0.08
	40	5.610 ± 0.12	10.76 ± 0.15
	50	7.120 ± 0.11	11.37 ± 0.17

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

Table 6B Phytoremediation (uptake) of heavy metals (Cd, Cu, Ni, Pb and Zn) by sunflower in contaminated soil. [†]

Metal	Dose (ppm)	Sunflower	
		Roots metal uptake (ppm)	Shoots metal uptake (ppm)
Cd	5	0.61 ± 0.01	1.10 ± 0.05
	10	1.60 ± 0.08	2.34 ± 0.17
	20	2.10 ± 0.05	4.81 ± 0.10
	40	4.16 ± 0.04	9.02 ± 0.16
	50	5.21 ± 0.12	11.52 ± 0.13
Cu	5	0.90 ± 0.04	1.61 ± 0.08
	10	1.32 ± 0.04	2.71 ± 0.03
	20	2.68 ± 0.05	5.27 ± 0.07
	40	5.02 ± 0.07	10.78 ± 0.08
	50	6.07 ± 0.07	13.16 ± 0.15
Ni	5	0.51 ± 0.04	1.31 ± 0.04
	10	1.27 ± 0.07	2.15 ± 0.02
	20	2.31 ± 0.04	4.70 ± 0.07
	40	3.96 ± 0.25	8.13 ± 0.12
	50	4.67 ± 0.09	12.15 ± 0.14
Pb	5	0.72 ± 0.03	0.28 ± 0.02
	10	1.00 ± 0.09	0.60 ± 0.04
	20	2.86 ± 0.06	0.97 ± 0.07
	40	4.78 ± 0.17	1.36 ± 0.07
	50	6.05 ± 0.11	1.96 ± 0.06
Zn	5	1.25 ± 0.04	2.97 ± 0.07
	10	2.01 ± 0.08	5.16 ± 0.09
	20	5.13 ± 0.06	9.08 ± 0.08
	40	8.01 ± 0.08	16.32 ± 0.21
	50	9.20 ± 0.16	20.81 ± 0.31

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

Table 6C Phytoremediation (uptake) of heavy metals (Cd, Cu, Ni, Pb and Zn) by sorghum in contaminated soil. [†]

Metal	Dose (ppm)	Sorghum	
		Roots metal uptake (ppm)	Shoots metal uptake (ppm)
Cd	5	0.91 ± 0.06	0.197 ± 0.07
	10	0.92 ± 0.06	0.237 ± 0.04
	20	2.78 ± 0.08	0.796 ± 0.07
	40	4.51 ± 0.07	2.014 ± 0.10
	50	7.10 ± 0.09	3.260 ± 0.14
Cu	5	1.66 ± 0.14	0.539 ± 0.05
	10	2.50 ± 0.12	1.063 ± 0.05
	20	2.59 ± 0.12	1.590 ± 0.11
	40	7.35 ± 0.14	1.828 ± 0.13
	50	12.66 ± 0.14	3.450 ± 0.13
Ni	5	1.23 ± 0.08	0.603 ± 0.08
	10	1.81 ± 0.19	0.817 ± 0.09
	20	5.14 ± 0.14	1.003 ± 0.08
	40	5.79 ± 0.17	1.319 ± 0.08
	50	6.27 ± 0.15	1.982 ± 0.11
Pb	5	0.60 ± 0.02	0.105 ± 0.02
	10	0.91 ± 0.05	0.237 ± 0.01
	20	2.20 ± 0.08	0.471 ± 0.02
	40	3.91 ± 0.11	0.534 ± 0.03
	50	6.07 ± 0.13	0.812 ± 0.08
Zn	5	3.10 ± 0.08	1.820 ± 0.05
	10	4.81 ± 0.12	2.488 ± 0.06
	20	9.45 ± 0.13	3.012 ± 0.12
	40	12.18 ± 0.18	5.693 ± 0.07
	50	12.61 ± 0.13	5.864 ± 0.09

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

within the plant. Among the three chosen plants, Alfalfa has a number of characteristics that make it a prime candidate for mitigation of environmental contamination problems. This includes deep rooted (commonly 270-480 cm) system, an active rhizosphere and its ability to absorb water, nitrates, and other heavy metals (Putnam 2001). Herbaceous plants accumulate higher concentration of metals and have higher bioconcentration factor in relative to woody plants (Lai and Chen 2009).

Baligar *et al*. (1993) recommended *M. sativa* (alfalfa) as a good source of plant tissues, because it has been found to tolerate heavy metals and grow well in contaminated soils. The higher concentrations uptake of heavy metals (Cu, Zn, Fe, Al, and Mn) by alfalfa (*M. sativa*) was reported by Rebah *et al*. (2002). Sunflower, on the other hand has reported to be the fast growing deep-rooted industrial oil crop with a high biomass producing plant species to remove heavy metals from contaminated environment (Prasad 2004). 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. The most sensitive species tolerated Zn additions of 200 to 300 ppm before undergoing a significant growth yield (Boawn and Rasmussen 1971).

The uptake of Pb by 5.6 mg in shoots and 61.6 mg in roots of sunflower plant has been evaluated by Nandakumar *et al*. (1995). The large surface area of fibrous roots of sorghum and intensive penetration of roots in to the soil reduces leaching via stabilization of soil (Jadia and Fulekar 2008a). The pot experiment conducted by Das and Maiti (2009) suggested that application of manure in combination with lemon grass, could be a viable option for reclamation (phytostabilization) of toxic metals (Mn and Zn).

The uptake of 8.2 mg of Pb by root of *Sorghum sp.* also has been documented. The higher concentrations of Pb in roots was reasoned out as lead might be bound to the outer surface of plant roots, as crystalline or amorphous deposits and could also be deposited in the cell walls or in vesicles (Nandakumar *et al*. 1995). Influences of vermicompost on uptake of heavy metals (Ni, Cr and Cd) by chamomile (*Matricaria chamomilla*) were evaluated by Chand *et al* (2008). Results indicated that there was significant effect of integrated supply of vermicompost and heavy metals on fresh herb (stem and leaves), flower and root yield of chamomile.

Results obtained in this research work showed that the three plant species tested, have potential for use in phytoremediation (uptake) of heavy metals. Sunflower, however is the best meet to the prerequisites for uptake and removal of heavy metals from the soil and thus would prove to be the most potent plant for phytoremediation of heavy metals among three plants considered for this study. The green plants used for phytoremediation of heavy metals have been found to have high potential in vermicompost –soil media pot culture experiment. The laboratory study explored here can be applicable to the field to remediate the heavy metals contaminated environment by application of vermicompost in soil using green plants.

CONCLUSION

The present research study has high significance to phytoremediation of the heavy metals from contaminated sites by application of the vermicompost to soil and growing the green plants at their highest tolerable concentrations. The vermicompost developed from vegetable waste had high nutrient contents and therefore it could be used as a natural fertilizer to increase uptake of heavy metals by the plants that plays a key role in phytoremediation. Sunflower, however best meets with the prerequisites for uptake and would have the potential for use in phytoremediation of heavy metals. Overall, heavy metals (Cd, Cu, Ni, Pb and Zn) accumulation potential of sunflower was found greater than that of sorghum and alfalfa as represented in the results. In the field, the plants can be harvested after their sufficient growth in vermicompost-amended contaminated soil further burnt to ash for recovery of heavy metals on its safe disposal in the environment. The present research findings can be explored for field experiment to clean up the heavy metals contaminated environment.

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