African Journal of Biotechnology Vol. 12(43), pp. 6185-6192, 23 October, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2246
ISSN 1684-5315 ©2013 Academic Journals

Full Length Research Paper

Phytoremediation of heavy metal contaminated soil using different plant species

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Accepted 24 September, 2012

A pot experiment was conducted to compare the plant biomass accumulation and heavy metal (HM) uptake by plant species grown in HM contaminated soils. The shoot dry weights of *Eucalyptus camaldeulensis*, *Medicago sativum*, and *Brassica juncea* grown in contaminated soils were reduced by 8, 5, and 3-fold, respectively, compared to the same plants grown in control soil. The Pb concentration in the shoots of *M. sativum, E. camaldeulensis* and *B. juncea* grown in contaminated soil was 8.7, 11.0, and 8.8-fold, respectively, higher than Pb concentration in plants grown in control soils. *M. sativum and E. camaldeulensis* accumulated higher Zn concentrations in roots (71 and 86 mg kg^{-1}) and shoots (49 and 47 mg kg^{-1}), respectively. Zn concentrations in the roots of *M. sativum, E. camaldeulensis* and *B. juncea* were higher than in the shoots by a factor of 1.4, 1.8, and 1.3-fold, respectively. The highest Cu concentration (81 and 37 mg kg^{-1} dwt) was obtained in root and shoot of *M. sativum* grown in contaminated soil, while the highest Cr concentration (133.9 mg/kg dwt) was determined in the root of *E. camaldeulensis*. This suggests that *E. camaldeulensis* was the best candidate species for phytoremediation of HM contaminated soils.

Key words: Keywords: Phytoextraction, Roadside soil, Heavy metal, *Brassica juncea*, *Medicago sativum*, *Eucalyptus camaldeulensis*

INTRODUCTION

Heavy metals (HMs) in roadside soils may come from various human activities, such as industrial and energy production, construction, vehicle exhaust, waste disposal, as well as coal and fuel combustion (Li et al., 2001; Bai et al., 2008). A large number of sites worldwide are contaminated by HMs as a result of human activities. HM contamination of soil requires effective and affordable remediation technologies. Unlike organic pollutants, metals cannot be degraded to harmless products, such as carbon dioxide, but instead persist indefinitely in the environment complicating their remediation (Lasat, 2002). Present technologies rely upon metal extraction or immobilization processes, although both are expensive and result in the removal of all biological activity in the soil during decontamination. Other, metal-extraction processes use stringent physicochemical agents that can dramatically inhibit soil fertility with subsequent negative impacts on the ecosystem (Wenzel et al., 1999).

The use of plants for rehabilitation of polluted environments is known as phytoremediation. This technology was developed after the identification of certain plants, metal “hyperaccumulators”, that are able to accumulate and tolerate extremely high concentrations of metals in their shoots (Chaney, 1983; Baker et al., 2000). A few plant species are able to survive and reproduce on soils heavily contaminated with Zinc (Zn), Copper (Cu), Lead (Pb), cadmium (Cd), Nickel (Ni), Chromium (Cr), and Arsenic (As) (Baker, 1987). Such species are divided into two main groups: the so-called pseudometallophytes that grow on both contaminated and non-contaminated soils, and the absolute metallophytes that grow only on metal-contaminated and

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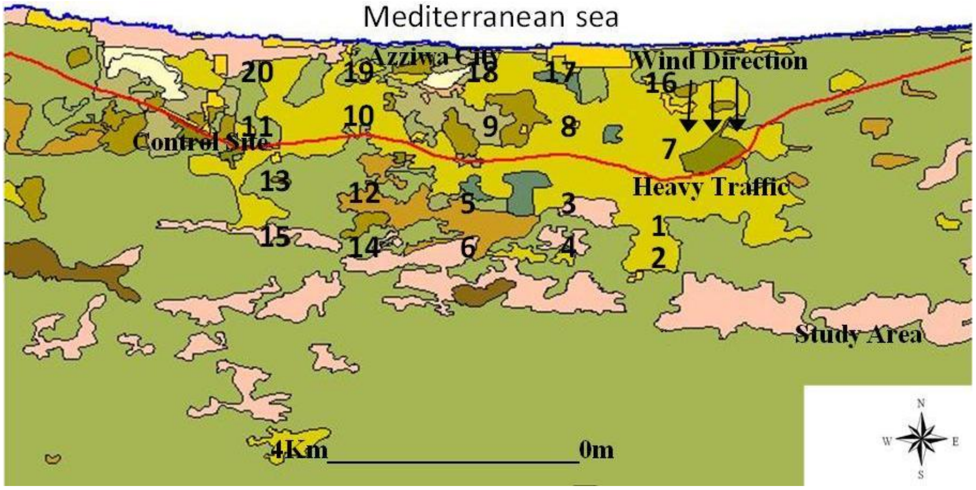

Figure 1. Location of soil sampling sites.

naturally metal-rich soil (Baker, 1987). Phytoextraction technologies are based on the use of hyperaccumulator plants with exceptional metal-accumulating capacity. These plants have several beneficial characteristics such as the ability to accumulate metals in their shoots and an exceptionally high tolerance to HMs (Baker et al., 2000). Baker and Brooks (1989) defined hyperaccumulator plant species as plants which accumulate >1000 mg/kg of Cu, Cobalt (Co), Cr, Ni or Pb, or >10,000 mg/kg of Manganese (Mn) or Zn. Hyperaccumulation of metals has been found in temperate as well as in tropical regions throughout the plant kingdom, but is generally restricted to endemic species growing on mineralised soil and related rock types (Baker and Brooks, 1989).

The capacity to specifically accumulate high amounts of metals in shoots makes hyperaccumulators suitable for phytoremediation purposes. However, various practical drawbacks can reduce the applicability of phytoremediation (Ernst, 1996). Crops with both a high metal uptake capacity and a high biomass production are needed to extract metals from soils within a reasonable time frame (Ebbs and Kochian, 1997; Abou-Shanab et al., 2008). Therefore, the objective of this study was to compare the uptake of Pb, Zn, Cr, and Cu by *E. camaldeulensis*, *B. juncea* and *M. sativum* grown in HM contaminated and uncontaminated soils. The potential use of these species in the phytoremediation of Pb, Zn, Cu and Cr polluted road side soils was also assessed.

MATERIALS AND METHODS

Sampling sites

Twenty sites were selected for the study along the length of the road connecting Triopli, with the southern parts of Libya. These city was chosen based on the high traffic density. Soil samples were collected at different distances from the edge of the main road (3 and 10 m) on both sides, north and south of the road. The distances between each site were about 1 km along the road (Figure 1).

Soil sampling and preparation

Samples were collected in summer to avoid rain washing out the HMs. Five samples of topsoil (0 to 10 cm) depth were collected from each site at the above mentioned distances, with a stainless steel trowel. Soils were mixed in a large container and dried at room temperature, then crushed by hand, sieved through a 4 mm stainless steel sieve to remove rocks and un-decomposed organic materials. Soil mechanical analysis was carried out by the pipette method according to Balck et al. (1982). The percentage of water-holding capacity (WHC) was determined according to the study of Alef and Nannipieri (1995). A glass electrode pH meter and conductivity were used for the determination of soil pH and conductivity. The electrode was immersed in the soil paste with a soil : water ratio of 1:2.5, to avoid errors due to higher dilution (Black et al., 1982). Organic carbon content was measured by the rapid titration method (Nelson and Sommers, 1986).

Cation exchange capacity (CEC) was determined by the method of Thomes (1982). Total nitrogen in soil samples was determined using the Kjeldahl method (Nelson and Sommers, 1980; Jones et al., 1991). Total metals in soil were determined by digesting 0.5 g soil in a mixture of concentrated HNO_3/$HClO_4$ (10: 7, V: V) (McGrath and Cunliffe, 1985), and the water extractable soil metals done by using $CaCl_2$. The total and extractable metal in soil samples were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) and flame atomic absorption spectrophotometry (AAS).

Pot experiment

To initiate the experiments, about 2 kg of air-dried soils were collected from site number 1 and 2 (Fig. 1) placed into plastic pots (18 cm in diameter and 13 cm in length). Seeds of *B. juncea, M. sativum* and *E. camaldeulensis* were sown in plastic pots which contains heavy metals contaminated soil (site number 1 and uncontaminated soil (site number 13) with four replicates for each treatment. The experiment was carried out in a greenhouse illuminated with natural light. The moisture content of each pot was maintained at 70% WHC by weighing the pots two times per week.

Table 1. Total metal contents and $CaCl_2$ extractable metals (mg/kg) of the studied soils.

Sampling site	Profile (cm)	Co		Cr		Cu		Ni		Cd		Pb		Zn	
		T	E	T	E	T	E	T	E	T	E	T	E	T	E
		Mg/kg dry soil													
Site No. 1 (3 m S)[a]	0 - 10	2	0.02	11	0.12	26	0.30	3	0.03	0.6	0.01	840	8.8	67	0.79
Site No. 13 (3 m S)[b]	0 - 10	0.5	0.01	2.9	0.03	25	0.29	2.1	0.02	0.08	ND	24	0.2	15	0.2

[a]Collected from 3 m south away from roadside; [b]collected from 3 m south away from roadside, T, Total metal; E, extractable metal.

After germination, the seedlings were thinned to two plants per pot and grown for eight weeks.

Plant harvest and analysis

After 8 weeks, plants were gently removed from the pots. Shoot and roots were separated and the lengths of both were measured. Plant shoots and roots were washed with deionized water, rinsed, and dried at 70°C, and the dry matter (DM) measured. Plant materials were ground and 2 g or less of milled plant matter was digested with 3 a mixture of HCl/HNO (4:1, v/v) (McGrath and Cunliffe, 1985), and the HMs (Zn, Cu, Cr and Pb) in the digests were determined using flame atomic absorption spectrophotometry (AAS).. Data were analyzed using Minitab (Version 16 English), significant between variables and were tested by analysis of variance (ANOVA) and means separated by using a Tukey test P ≤ 0.05.

RESULTS AND DISCUSSION

Physical and chemical characteristics of soil samples

All soils were found to be granular with a sandy texture. Sandy soils are generally poor in nutrient reserves and have low water holding capacity (WHC). Soil moisture influences the chemistry of contaminated soil. Drainage moisture control can influence micronutrient solubility in soils. The moisture content and water holding capacities in soil samples were generally low and ranged from 0.1 to 0.5% and from 7.40 to 9.41%, respectively. Soil pH is one of the most influential variable controlling the conversion of metals from immobile solid-phase to more the mobile and/or bioavailable solution phase .Soils site had a pH of from the (7.5-8.5).

The solubility of heavy metals is generally greater in the pH range of normal agriculture soils. The organic matter (OM) content varied between 0.05% and 0.09%, and cation exchange capacity (CEC) in most of the samples ranged from 7.63 to 9.10 meq 100 g/soil. The behaviour of trace metals in soils depends not only on the level of contamination as expressed by the total metal content, but also on the form and origin of the total metal and the properties of the soils themselves (Tessier and Campbell, 1988; Chlopecka et al., 1996). The physical and chemical properties of the soil influence the form of the metal contaminant, its mobility, and the technology selected for remediation (Gerber et al., 1991). OM and pH are important factors that control the availability of HMs in the soil (Karaca, 2004). In general, soils with high CEC can adsorb larger amounts of HMs than soils with low CEC (Singh et al., 2001).

The total metal content is important because it determines the size of the metal pool in the soil and is thus available for metal uptake (Ibekwe et al., 1995). Therefore the soil samples were analyzed for total and $CaCl_2$ extractable concentrations of Co, Cr, Cu, Mn, Molybdenum (Mo), Ni, Fe, Cd, Pb, and Zn. Results showed that each site exhibited a high concentration of one or two metals. Variation was also recorded in the extractable metal content, that is, biologically available metals in comparison to the total metal content in the same soil. This can be attributed to the behaviour of trace metals in soils that depends not only on the level of contamination, as expressed by the total content, but also on the form and origin of the metal and the properties of the soils themselves (Chlopecka et al., 1996). The bioavailable concentrations of metals extracted by 0.5 M $CaCl_2$ extraction were found to be lower than the total concentration, because there are many factors affecting trace element availability in soils including physical and chemical properties (Alloway, 1995; Pueyo et al., 2003).

The highest values of metal (Pb) were recorded in soil samples collected from 3 m south of the roadside (Table 1). Pb, the element of most concern in environmental HM pollution in Libya, exhibited high levels of contamination closer to highway. Since the fuel used by automobiles in Libya is mostly leaded, the most probable source of such contamination is the Pb particulate matter emitted from gasoline which settles not far from the highway (Harrison and Laxen, 1981). Similar results were found by other investigators (Ho and Tai, 1988; Culbard et al., 1988). As the distance from the road increased, the Pb level decreases. The maximum Pb concentration (840 mg/kg) was detected in a soil sample collected at 3 m south of the road. Therefore, the soil collected from this site was selected to phytoremediate using different plant species.

Effect of heavy metal contaminated soil on plant biomass

The plant species (*B. juncea, M. sativum,* and *E. camaldeulensis*) were chosen for this study based on their high biomass, and their ability to remove heavy

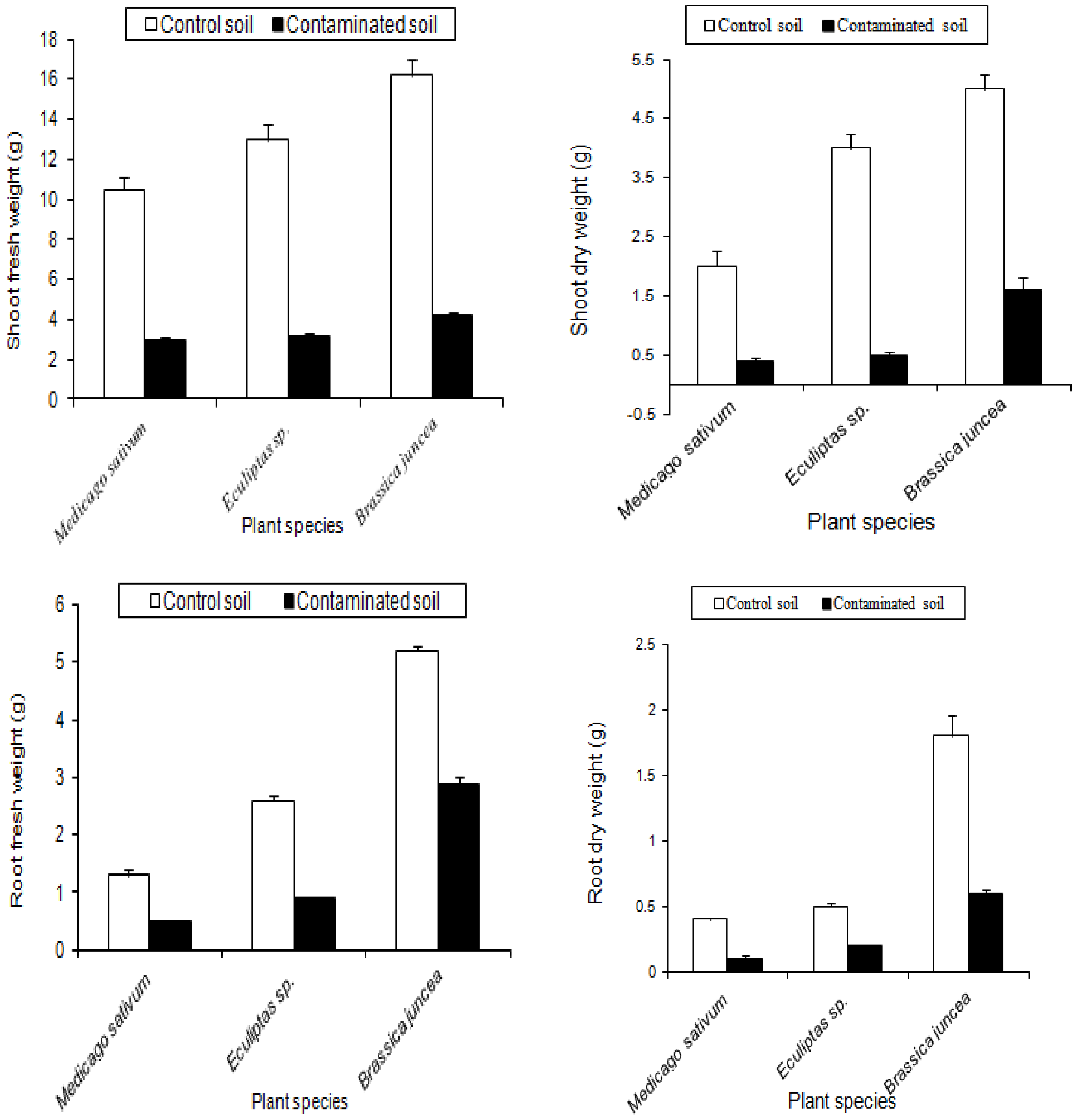

Figure 2. Shoot and root dry and fresh weights of plant species grown in a greenhouse using soil collected from two different sites. Mean values marked with the same letter are not significantly

metals from contaminated sites (Beladi et al., 2011; Turan and Esringu, 2007; Waranusantigul et al., 2011). All three plant species appeared healthy in the low (control) and high Pb contaminated soils. In this experiment, there were higher differences between the three plant species in shoot, root dry and fresh weights (Figure 2). The root dry weights were reduced in all plants grown on Pb contaminated soils compared with the same plants grown on control soils. The root dry weight in *M. sativum, B. juncea* and *E. camaldeulensis* grown in Pb contaminated soils, were reduced by 4, 3 and 2.5-fold lower than the root dry weight of the same

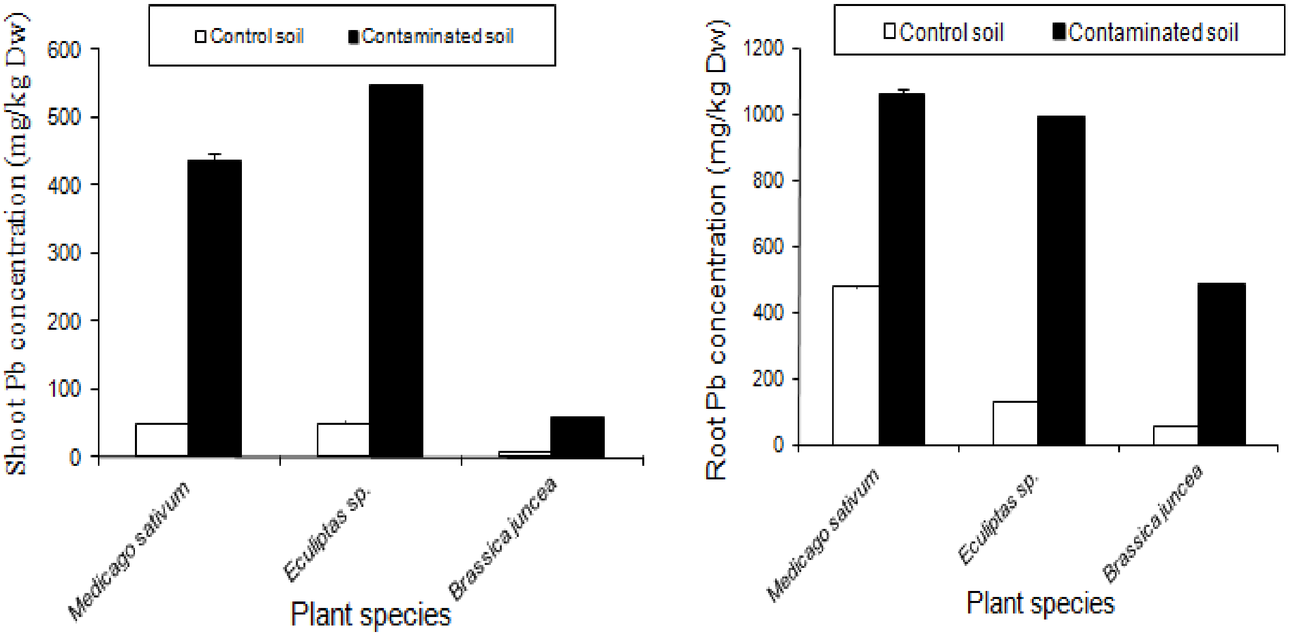

Figure 3. Concentration of Pb in shoot and root of different plant species grown in a greenhouse using soil collected from two different sites. Mean values marked with the same letter are not significantly different P < 0.05.

plants grown on the control soil, respectively (Figure 2). The shoot dry weights of *E. camaldeulensis, M. sativum* and *B. juncea* grown on Pb contaminated soils were also reduced by 8, 5 and 3-fold, respectively compared to the same plants grown in the control soil.

The shoot and root fresh weights were also reduced by 3.4, 4 and 3.8-fold and 2.6, 2.8 and 1.8-fold, in *B. juncea*, *M. sativum,* and *E. camaldeulensis*, respectively grown in Pb contaminated soils compared with the same plant species grown in the control soils (Figure 2). Turan and Esringu (2007) reported that the total dry weight of *B. juncea* and canola were affected by the contaminated soil; on average, the metals caused a reduction of about 75% in root and shoot DM of both plants. Similar results in other hyper-accumulator plants were reported by Chen and Cutright (2001), Hajiboland (2005) and Tlustos et al. (2006).

Heavy metal uptake by plant species

Metal concentrations in plant tissues also differed among the three plant species grown on the same soils, indicating their different capacities for metal uptake. Pb concentrations in roots of plants were elevated and varied from 55.8 to 1058 mg/kg dry weight (Dw). The highest concentrations of Pb (548 mg/kg) were found in the shoots of *E. camaldeulensis* grown in Pb contaminated soil. Pb concentrations in the root and shoot of *E. camaldeulensis* grown in contaminated soil was about 11 and 7.5-fold, respectively higher than Pb concentration in the root and shoot of the same plant grown on the control soil. However, the Pb concentration in the roots of *M. sativum, E. camaldeulensis*, *B. juncea,* grown in the Pb contaminated soil was 2.2, 7.5 and 8.6-fold higher than Pb concentrations in the roots of the same plant species grown on control soils (Figure 3), while, the Pb concentration in the shoots of *M. sativum* and *E. camaldeulensis*. *B. juncea* grown on the Pb contaminated soil was 8.7, 11.0 and 8.8-fold higher than Pb concentration in the same plant species grown on control soils. Our results indicated that the highest Pb concentration (1058 and 548 mg/kg) was found in the root of *M. sativum* and shoot of *E. camaldeulensis*, respectively grown in Pb contaminated soil (Figure 3). *M. sativum* and *E. camaldeulensis* accumulated higher Zn concentrations in roots (71 and 86 mg/kg) and shoots (49 and 47 mg/kg), respectively than Indian mustard grown in the same soil. Zn concentrations in the roots of *M. sativum*, *E. camaldeulensis* and *B. juncea* were higher than in the shoots by a factor of 1.4, 1.8 and 1.3-fold, respectively (Figure 4). As shown in Figure 5, the highest Cu concentration (81 and 37 mg/kg Dw) was obtained in root and shoot of *M. sativum* grown on Pb contaminated soil. The highest Cr concentration (133.9 mg/kg Dw) was determined in the root of *E. camaldeulensis* grown on contaminated soil (Figure 6).

Accumulation and translocation of metals in plants

The mobility of the HMs from the polluted substrate into the roots of the plants and the ability to translocate the metals from roots to the harvestable part of the plant

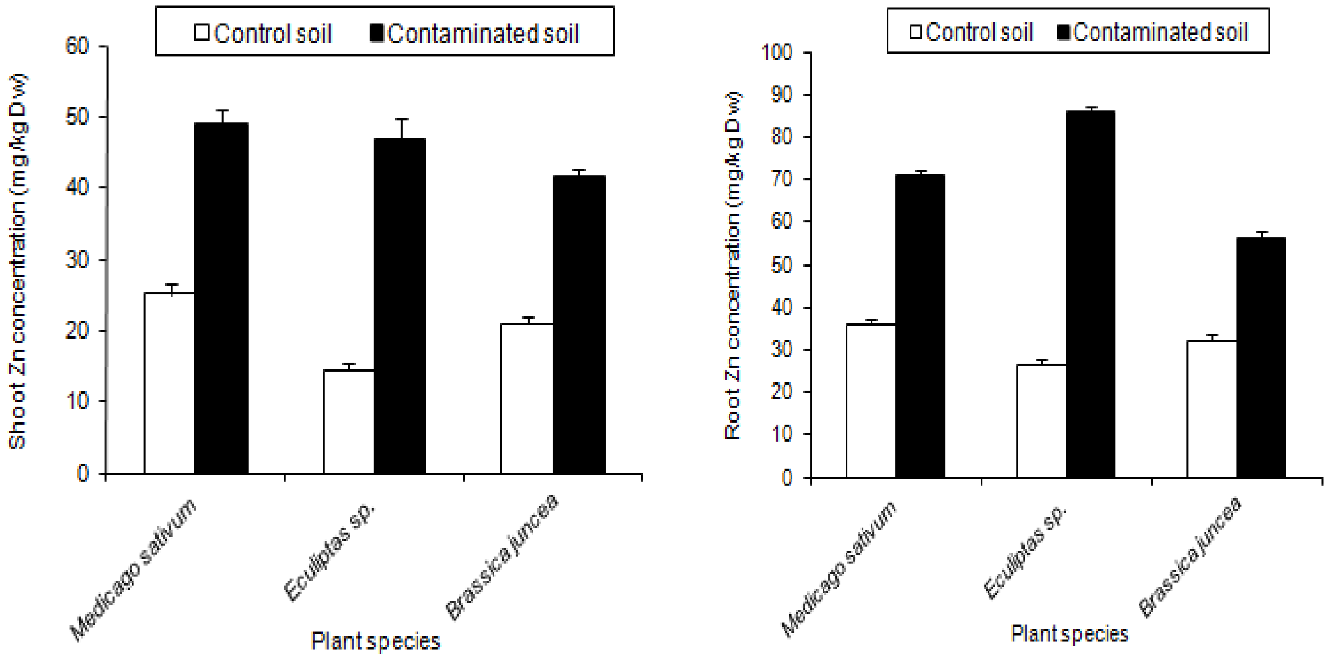

Figure 4. Concentration of Zn in shoot and root of different plant species grown in a greenhouse using soil collected from two different sites. Mean values marked with the same letter are not significantly different P < 0.05.

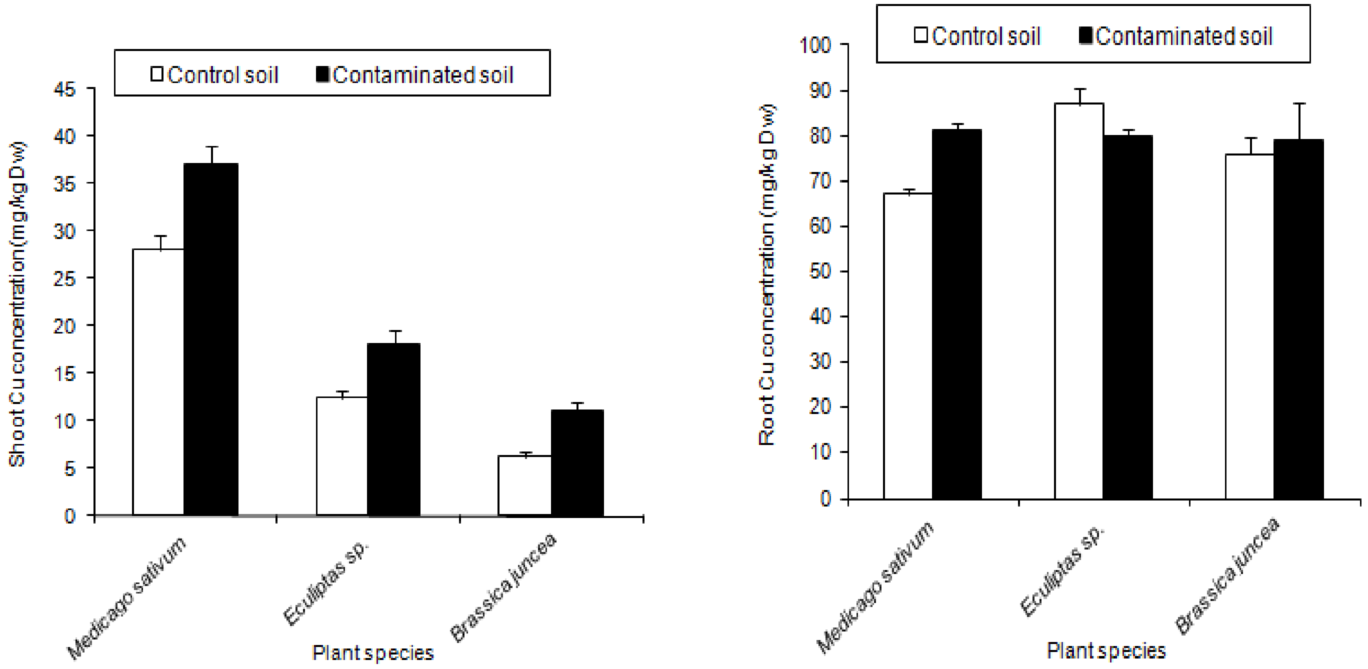

Figure 5. Concentration of Cu in shoot and root of different plant species grown in a greenhouse using soil collected from two different sites. Mean values marked with the same letter are not significantly different P < 0.05.

were evaluated, respectively by means of the bioconcentration factor (BCF) and the translocation factor (TF). BCF is defined as the ratio of metal concentration in the roots to that in soil ([metal]root/ [metal]soil). TF is the ratio of metal concentration in the shoots to the roots ([Metal]Shoot/[Metal]Root). The ability of plants to tolerate and accumulate HMs is useful for phytoextraction and phytostabilization purposes (Yoon et al., 2006). Plants with both BCFs and TFs greater than one (TF and BCF> 1) have the potential to be used in phytoextraction.

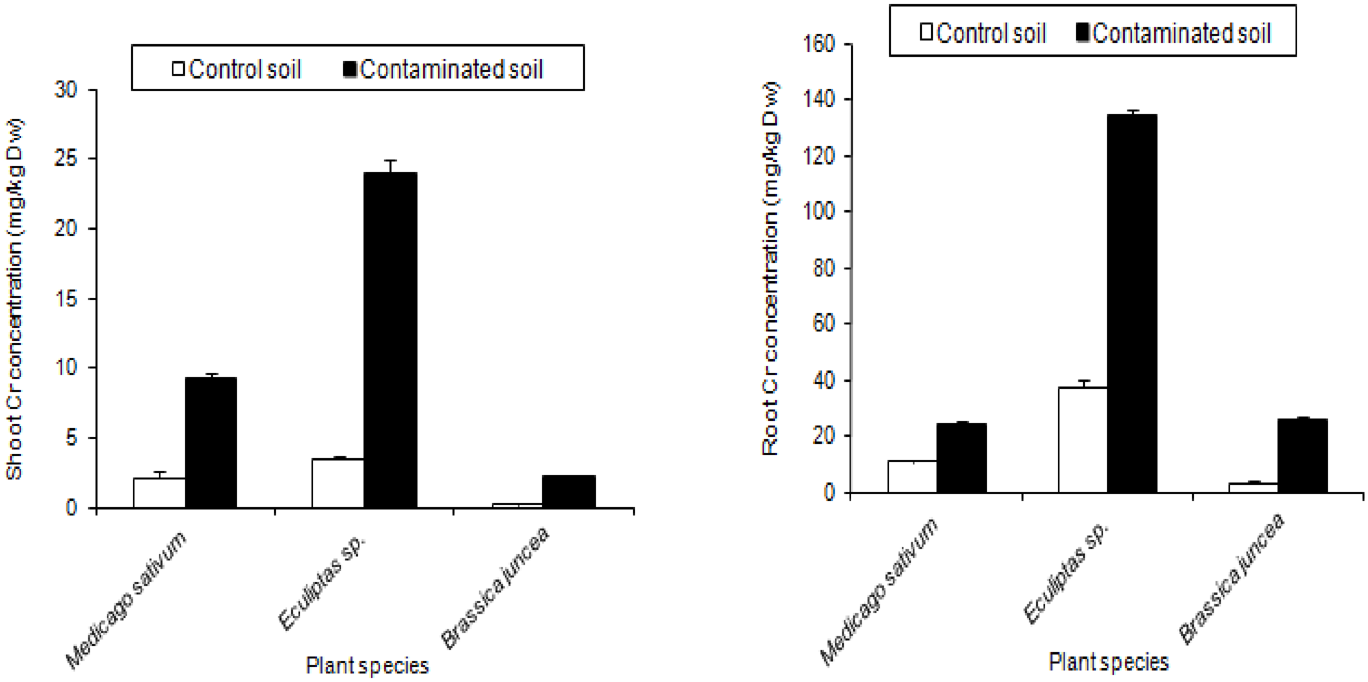

Figure 6. Concentration of Cr in shoot and root of different plant species grown in a greenhouse using soil collected from two different sites. Mean values marked with the same letter are not significantly different P < 0.05.

Table 2. Accumulation and translocation of Pb, Zn, Cu and Cr in plant species grown in metal contaminated soil.

Plant species	Bioconcentration factor (BCF)				Translocation factor (TF)				Enrichment factor (EF)			
	Pb	Zn	Cu	Cr	Pb	Zn	Cu	Cr	Pb	Zn	Cu	Cr
M. sativum	1.4	1	2.7	2	0.9	1.3	0.4	0.4	0.5	0.7	1.1	0.8
E. camaldeulensis	1.1	1.2	3	11	4.1	1.7	0.2	0.1	0.6	0.7	0.7	2.1
B. juncea	0.5	0.8	2.8	2.2	0.9	1.3	0.1	0.08	0.1	0.6	0.1	0.2

*BCF is calculated by relation:- ratio of metal concentration in the roots to that in soil ([Metal]Root/ [Metal]Soil), TF is given by relation:- the ratio of metal concentration in the shoots to the concentration of metal in the roots ([Metal]Shoot/[Metal]Root) and EF is calculated by relation:- the ratio of the concentration of metal in the shoots to the concentration of metal in the soil (Yoon et al., 2006).

Besides, plants with BCF greater than one and TF less than one (BCF> 1 and TF< 1) have the potential for phytostabilization (Yoon et al., 2006). A plant's ability to accumulate metals from soils can be estimated using the BCF and a plant's ability to translocate metals from the roots to the shoots can be measured using the TF. The process of phytoextraction generally requires the translocation of HMs to the easily harvestable plant parts, that is, shoots (Yoon et al., 2006), while phytostabilization process requires the strong ability to reduce metal translocation from roots to shoots (Deng et al., 2004).

By comparing BCF and TF, the ability of different plants in taking up metals from soils and translocating them to the shoots can be compared (Yoon et al., 2006). Among tested plant species, *E. camadulensis* was suitable for phytoextraction of Pb and Zn, while *M. sativum* and *B. juncea* were suitable for phytostabilization of Cu and Cr (Table 2).

Conclusion

Phytoremediation is an emerging technology for the remediation of metal contaminated soils requiring more of hyperaccumulator species, especially for specific metals. The result of this study generally revealed the metal concentrations in the roadside soils at this site, and are in the order of Pb>Zn>Cu>Cr. The level of Pb was high compared with other studies. Accumulation of metal in the soil and subsequent transfer to plants growing along the edge of the road could occur as a result of continual usage of the road by automobiles research in to the patterns of Pb, Zn, Cu and Cr accumulation in *B. juncea*, *M. sativum* and *E. camaldeulensis* growing in Pb contaminated soils revealed accumulation of Pb, Zn, Cu, and Cr in significantly different quantities in their shoots and roots. Considering the TF and BCF, *E. camadulensis* was suitable for phytoextraction of Pb and Zn, while *M. sativum* and *B. juncea* were suitable for phytostabilization of Cu and Cr.

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