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[Research]

Bioaccumulation of Nickel and Lead by Bermuda Grass (*Cynodon dactylon*) and Tall Fescue (*Festuca arundinacea*) from Two Contaminated Soils

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ABSTRACT
Soil and sediments of the estuaries and wetlands in Northwest of Persian Gulf are recently polluted with different heavy metals because of municipal and industrial wastewaters. Therefore an urgent soil cleaning up and remediation program is vital in this region. Consequently, this study was initiated to screen two plant species (*Festuca arundinacea* and *Cynodon dactylon*) for hyperaccumulation of nickel (Ni) and lead (Pb) as one of the candidate methods for cleaning-up soil and sediments of Shadegan wetland. Soil samples (0-30 cm) were collected from two sites in the wetland. The soil samples were treated with solutions of Ni and Pb separately which resulted into content of 50 and 100 mg kg^{-1} of metals in each soil. Thereafter, the plants were sown in the soils under greenhouse conditions and harvested after 10 weeks. Ni and Pb contents were measured in root and shoot of plants. Results showed that accumulation of Ni and Pb in tall fescue roots were significantly ($P<0.05$) greater than that in Bermuda grass. The amounts of Pb in root and shoot of plants were increased when soil Pb contents were increased from 50 to 100 mg kg^{-1} while Ni contents were only increased in the roots in response to increase in soil Ni content. The comparing of the shoot-root ratio showed that Pb accumulation in the roots of both plants was higher than that in the shoots, while for Ni was reverse. Due to difference in backgrounds of soil metal contents and soil characteristics, accumulation of Ni and Pb by plants were different in two soils

Keywords: DTPA-extraction, Heavy metals, Hyperaccumulator, Phytoavailability, Phytoremediation, Pollution.

INTRODUCTION
Soils contaminated with heavy metals pose a risk to soil quality and human health in southwestern Iran. Shadegan wetland as the largest wetland in Iran covering about 400,000 hectares, is an important ecosystem in the region contaminated with nickel (Ni), lead (Pb), vanadium (V) and cadmium (Cd) due to dumping municipal and industrial wastewaters (Nouri *et al*.,2009). Conventional clean up technologies for restoration of contaminated soils such as vitrification and chemical treatments are generally costly and restricted to relatively small areas (Mulligan *et al*., 2001). It is necessary to remediate the contaminated soils in this area with gentle methods. Phytoremediation, i.e. the use of plants to remediate polluted sites, has been mentioned as a suitable alternative to traditional methodologies (Glass, 2000; Chaney *et al*., 1997). However, the metal uptake by plants and transfer to a higher trophic level by means of herbivores is a disadvantage of this method that should be taken into account (Beeby 1985). Phytoremediation is recommended for sites contaminated with heavy metals in several ways. Those may include phytoextraction, where metals are transported from soil into harvestable shoots (Salt and Blaylock, 1995); rhizofiltration, where plant roots or seedlings grown in aerated water precipitate and concentrate toxic metals (Raskin *et al*, 1997); phytovolatilization, in which plants extract

volatile metals (e.g. mercury and selenium) from soil and volatilize it from the foliage (Salt and Blaylock, 1995) and phytostabilization, in which metal-tolerant plants are used to reduce the mobility of heavy metals (Raskin *et al*., 1997). Using the metal-accumulating plants to remove metals from contaminated soils has been proposed because of environmental-friendliness and cost benefits of phytoremediation (Cunningham *et al*., 1995). The concept of using plants for cleaning contaminated sites, however, is not new. About 300 years ago, plants were introduced to treatments of wastewater (Hartman, 1975). Wild grasses can be suitable to reduce metal pollution in contaminated soils (McLaughlin *et al*., 2000). Many researches showed that Bermuda grass (Cynodon dactylon) as a widespread creeping grass could be useful for stabilizing spill-affected soils (Smith et al., 1998; Madejon *et al*., 2002). Begonia et al. (2005) reported that tall fescue was relatively tolerant to moderate levels of Pb in soil by non-significant differences in root and shoot biomass. Tall fescue was identified as a potential phytoextraction species because of its high biomass yield under elevated Pb levels and its ability to translocate high amount of Pb from roots to shoots (Begonia *et al*., 2001). Gawronski et al. (2002) studied 21 varieties of grasses including genera Festuca, Agrostis and Lolium and concluded that *Agropyrone repens* is the most promising plant for phytoremediation purposes as its high biomass of 50 t ha^{-1} can uptake of 20 kg of Pb from a soil.

The objectives of this research were *i*) to assess the soil phytoavailability and movement of Ni and Pb within selected plants for evaluation of phytoremediation potential, *ii*) to investigate the ability of Bermuda grass and tall fescue to accumulate lead and nickel after growing in two contaminated soils from Shadegan wetland, *iii*) to compare Ni and Pb accumulation by plants from two soils with different background metal contents.

Bermuda grass (*Cynodon dactylon*) and tall fescue (*Festuca arundinacea*) were selected because they have extensive rooting system and often are used for soil stabilization (Rizzi *et al*., 2004; Smith *et al*., 1998).

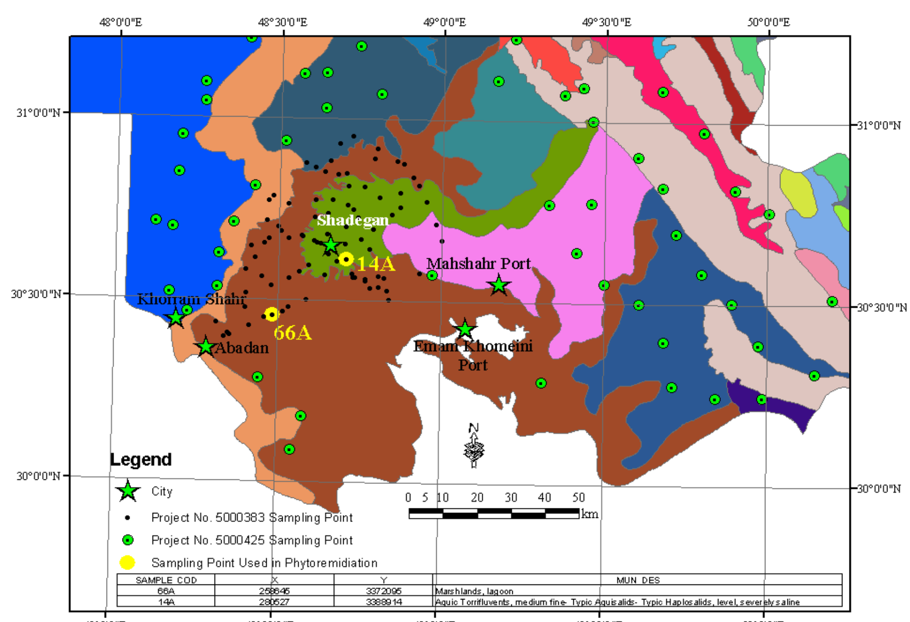

Fig 1. Map of the study area in northwest region of the Persain Gulf. The sampling sites are shown as 14A (Soil 1) and 66A (Soil 2). Major cities are also shown.

MATERIALS AND METHODS

Study Site and Soil Collection

Shadegan wetland is located in the northwest part of Persian Gulf (Fig. 1). It is the largest wetland area in Iran covering about half a million hectares of Jarahi River Basin. Soil samples for pot experiment were collected from sites 66A and 14A as shown in Figure 1. Some of soil characteristics were quantified in laboratory. Organic carbon was measured with Walkley and Black method (ISRIC, 1986). Total nitrogen and available phosphorous content were measured using the Kjeldahl steam distillation and Olsen methods, respectively (ISRIC, 1986). Soil pH and electrical conductivity (EC) were measured for all samples. Soil texture was determined by pipette method (ISRIC, 1986). Selected soil physical and chemical properties are shown in Table 1.

Table1. Selected physical and chemical properties of the soil samples (Mean± Standard Error).

Properties	Site 66A (Soil 1)	Site 14A (Soil 2)
pH	7.8±0.1	7.4±0.2
EC† (dS/m)	12±1.6	12.7±0.9
O.M†† (g kg^{-1})	8.7±1.3	11.1±1.5
Clay (g kg^{-1})	490±34.2	450±25
Silt (g kg^{-1})	360±23.3	410±37.4
Sand (g kg^{-1})	150±18.1	140±17
Total Pb (mg kg^{-1})	73±4.5	121.6±7.9
Total Ni (mg kg^{-1})	44.1±3.6	67±6.4
DTPA-extractable Pb (mg kg^{-1})	0.72±.08	1.74±0.1
DTPA-extractable Ni (mg kg^{-1})	0.98±0.6	2.18±0.2
CEC †††(cmol(+) kg^{-1}	9±1.9	12±1.7
Total N (%)	0.1±0.02	0.21±0.03
Total K (%)	0.35±0.1	0.39±0.08
Available P (mg kg^{-1})	140±13.2	260±25.3

† Electrical Conductivity
†† Organic Matter
†††Cation Exchange Capacity

Pot Experiments

The soil was rehydrated with nutrient solution (1000 ml kg^{-1} soil) containing 250 mg N (in the form of NH_4NO_3), 60 mg Mg (in the form of $MgSO_4$), 109 mg P (in the form of KH_2PO_4), and 207 mg K (in the form of KH_2PO_4+K_2SO_4) per liter to support plant growth (Brooks, 1977). Two days later, two experimental sets were started. One liter of each metal solution was spiked into one kg soil and mixed thoroughly. For the first experimental set, two solutions containing 50 mg l^{-1} Ni and 50 mg l^{-1} Pb were separately added to the soils in a 1:1 volume/weight ratio which plus background metal resulted in total Ni and Pb about 94-117 and 123-172 mg kg^{-1} in the soils, respectively. In the second experimental set, the soil samples were separately spiked with two solutions containing 100 mg l^{-1} Ni and 100 mg l^{-1} Pb which plus background metal resulted in total Ni and Pb about 120-170 and 170-230 mg kg^{-1} in the soils respectively. Then the soil samples were incubated for a period of 10 days in greenhouse. During this time and prior to mixing three cycles of saturation/drying with deionized water were performed (Bell and Failey, 1991). Each pot was filled with 2 kg of soil. The seeds of Bermuda grass (*Cynodon dactylon*) and tall fescue (*Festuca arundinacea*) were separately germinated in a sand bath using Hoagland nutrient solution. After germination, five similar seedlings were transplanted into each pot in a controlled–temperature greenhouse at 25 °C. Then seedlings were planted in the pots and watered based on 75 % of soil field capacity. Control pots (soil without metals addition) were also prepared with nutrient solution and saturation/drying cycles. Plants were harvested after 10 weeks and separated into shoots and roots and washed with deionized water.

Metal Determination

Soil samples were air dried and sieved through a 2 mm mesh sieve. Diethylene triamine pentaacetic acid (DTPA)-extractable heavy metals were measured by DTPA-TEA solution (Lindsay and Norwell, 1978). Extraction of soil heavy metals by DTPA is thought to give an estimate of the phytoavailable heavy metals (Haq *et al*., 1980). Soil total heavy metal contents were measured via an EPA acid digestion method 3050 (Carter, 1993). Ten ml of concentrated HNO_3 (65 %) was added to 1 g soil and heated for 10 min at 95±5 ^{o}C. After cooling, 10 ml H_2O_2 in 1-mL aliquots was added and heated for 6 min at 95±5 ^{o}C without boiling for additional two hours until the volume was reduced to approximately 5 mL. Finally, 10 ml of concentrated HCl was added to the solution and refluxed in 95±5 ^{o}C for 5 min and diluted to 100 ml with deionized water.

Before metal analysis for samples, plant roots were immersed in 0.01 M HCl for approximately 5 seconds in order to remove metal at the root surface. All plant samples (roots and shoots) were washed with deionized water, oven dried in a ventilated oven at 70 °C until no changes in weight were observed (Page, 1982). Plant tissues were digested following the procedure outlined by Zheljaskov and Erickson (1996). One gram of the milled plant material was soaked in 20 ml of concentrated nitric acid (65 %). After 6 h the mixtures were heated at 120 °C to 50 % of its original volume. Then, 4 ml of perchloric acid ($HClO_4$) was added and the mixtures refluxed for 90 min. The solution was finally diluted with deionized water to 25 ml of total volume and analyzed with flame atomic absorption spectrometry for Pb and Ni content.

Statistical Analysis

A completely randomized block design was implemented with two plants, two soils, two metals (Ni and Pb), three soil metal treatments (0, 50 and 100 mg kg^{-1}) and three replications. Analysis of variance procedure (ANOVA) for all treatments was conducted using SAS program (Release 8.02). The difference between specific pairs of mean was identified using Duncan test ($P<0.05$).

RESULTS

Soil Properties

Analysis for chemical and physical characteristics showed that both soils were within the ranges of fine texture and highly saline (Table 1). Soil 2 had a relatively higher amount of organic carbon and lower pH value as compared to soil 1, but pHs of both soils were near neutral to slightly alkaline. The main difference between these two soils was the variation in Pb, Ni, and P contents.

Nickel and Lead in Soil

Total and DTPA-extractable Ni content in soil 2 was higher than that in soil 1 (Table 1). Similar trends were seen for the lead, but overall DTPA-extractable Pb was lower than Ni. When Ni and Pb were added to the soils the amount of their DTPA-extractable forms were also increased as shown in Table 2. This increase was higher for Ni than Pb. The capacity of soil to adsorb Pb, increases with increasing pH, cation exchange capacity (CEC), organic carbon content, soil/water Eh (redox potential) and phosphate levels (EPA,1992).

Table 2. DTPA-extractable Pb and Ni of the collected soils (mg kg^{-1}) in different soil added metal treatments (control, 50 mg kg^{-1}, 100 mg kg^{-1}). Similar letters show insignificant difference ($P<0.05$).

	Soil 1		Soil 2	
	Ni	Pb	Ni	Pb
Before treatment	0.98±0.07e	0.72±0.1e	2.18±0.33d	1.74±0.15d
After treatment (50 mg kg^{-1})	15.4±1.06c	15.65±2.02c	24.08±2.35b	16.2±3.0c
After treatment (100mg kg^{-1})	34.75±3.07a	24.37±2.41b	35.1±2.93a	25.2±2.31b

Ni Accumulation by Plant Roots

Nickel accumulation by roots in tall fescue was significantly higher than Bermuda grass when the Ni content of soil increased from 50 to 100 mg/kg (Fig. 2). This shows the higher capability of tall fescue to accumulate Ni in roots as compared to Bermuda grass after 10 weeks planting. As the amount of Ni in soil increased, the bioaccumulation also increased due to higher phytoavailability of this element. A significant difference in Ni bioaccumulation by plants was also obtained though comparing the soil Ni treatments at 50 and 100 mg kg^{-1}. There was also significant difference between Ni bioaccumulation by the plant roots in two soils. For soil 2 the amount of Ni bioaccumulation by both plants was higher as compared to soil 1.

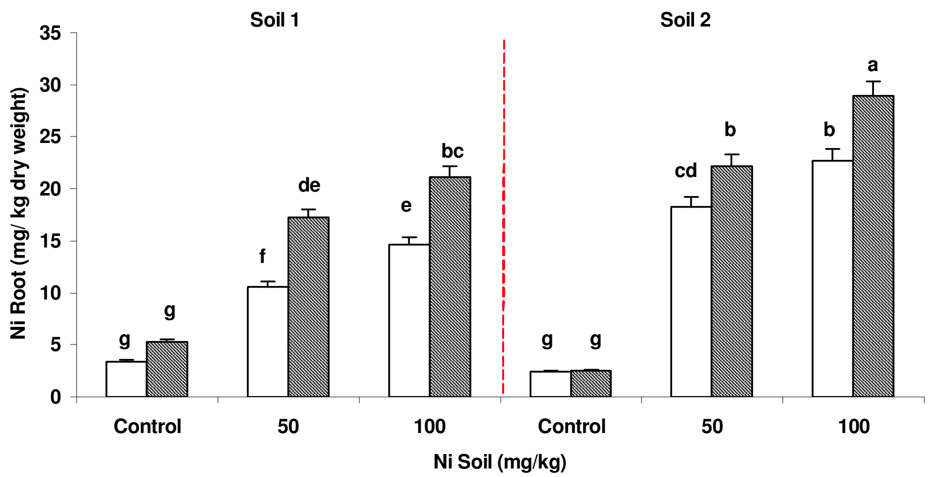

Fig 2. The Ni content in tall fescue (shaded) and Bermuda grass (unshaded) root with respect to soils with different levels of added Ni treatments (Control, 50 mg kg^{-1}, and 100 mg kg^{-1}). Bars with different letters represent significant difference (P<0.05).

Nickel Accumulation in Shoots

The amount of Ni accumulated in shoots of Bermuda grass was higher than tall fescue. However, this effect was significant only for soil 2 (Fig. 3). Tall fescue in soil 2 with 50 mg kg^{-1} Ni treatment had lower bioaccumulation than Bermuda grass. The 100 mg kg^{-1} Ni treatment resulted in a similar effect on the Ni accumulation by plants. Increasing soil Ni had no significant effect on the metal accumulation by plant shoots.

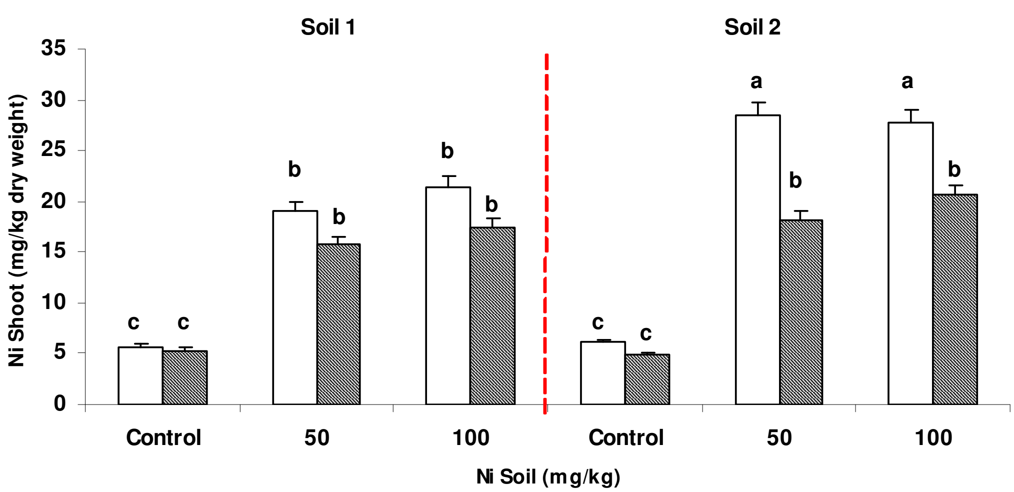

Fig 3. The Ni content in tall fescue (shaded) and Bermuda grass (unshaded) shoot with respect to soils with different levels of added Ni treatments (Control, 50 mg kg^{-1}, and 100 mg kg^{-1}). Bars with different letters represent significant difference (P<0.05)

Lead Accumulation in Roots

Results showed that Pb accumulation in roots was related to plant type, soil properties and the soil Pb content. Tall fescue accumulated more Pb in roots than Bermuda grass in both soils. Higher amount of Pb in the soils caused increasing of 5-8 times lead uptake by plant roots. Lead accumulation by plant roots in soil 2 was significantly higher than soil 1 (Fig. 4)

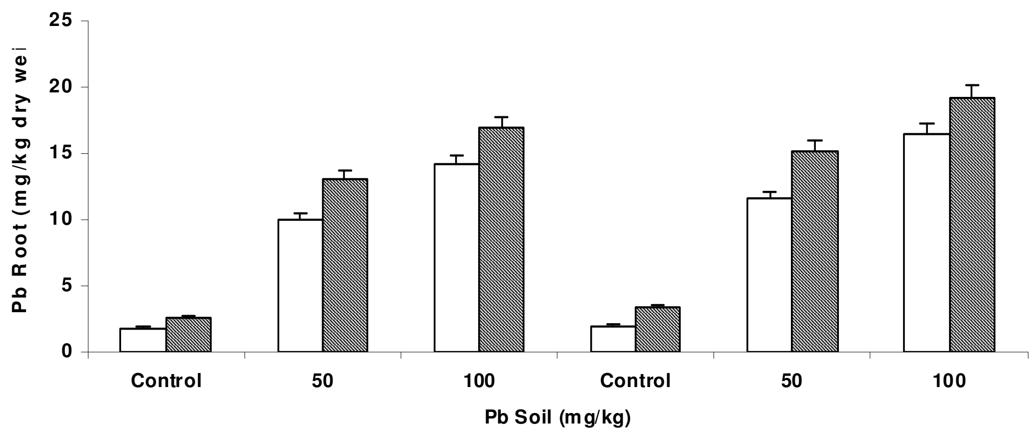

Fig 4. The Pb content in tall fescue (shaded) and Bermuda grass (unshaded) root with respect to soils with different levels of added Pb treatments (Control, 50 mg kg^{-1}, and 100 mg kg^{-1}). Bars with different letters represent significant difference (P<0.05).

Lead Accumulation in Shoots

As Pb concentration in soil increased, DTPA-extractable Pb in soil and translocation of this metal within the plant increased. Thus, Pb contents in shoots of both plants increased accordingly (Fig. 5). There was no significant difference in Pb accumulation in the shoots of tall fescue and Bermuda grass. Higher amount of soil Pb caused higher accumulation in plant shoots. Bioaccumulation of Pb by plant shoots in soil 2 was significantly higher than soil 1 in metal addition treatments (Fig. 5). The amount of accumulated Pb in shoots could be related to plant type, soil properties and soil Pb content (Begonia *et al*., 2005).

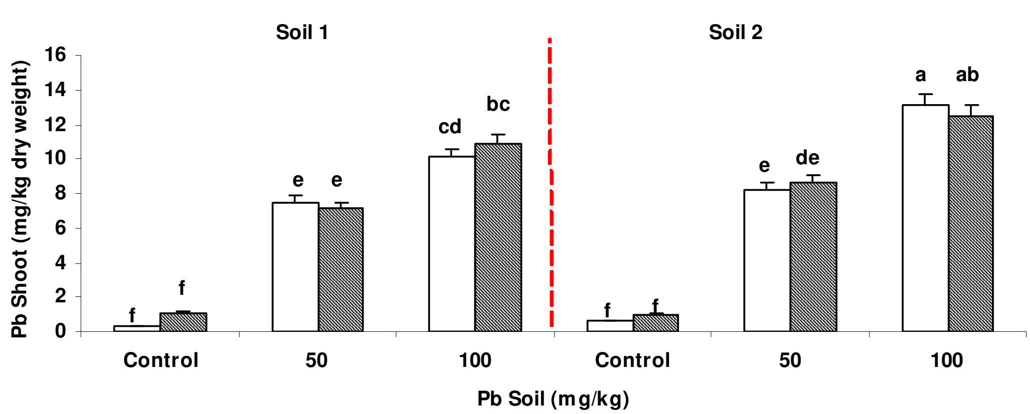

Fig 5. The Pb content in tall fescue (shaded) and Bermuda grass (unshaded) shoot with respect to soils with different levels of added Pb treatments (Control, 50 mg kg^{-1}, and 100 mg kg^{-1}). Bars with different letters represent significant difference (P<0.05).

Shoot-Root Ratio of Ni and Pb Accumulation

Shoot-root ratio of metals can help verifying translocation of these elements through the plant. All ratios were less than 1 for Pb indicating slow movement of Pb from root to the shoot (Table 3). Bermuda grass had higher capability in transferring Ni from root to shoot, where the shoot-root ratios of Ni in metal addition treatments were more than 1 and less than 1 for Bermuda grass and tall fescue, respectively (Table 4). It revealed that Bermuda grass had lower tendency in accumulating Ni in the roots, compared to tall fescue.

Plant Yield

Metals exposure inhibited dry weights of shoots and roots to be increased (Fig. 6). Root biomass in tall fescue of all treatments was higher than Bermuda grass (Fig. 6).

Table 3. Shoot to root ratio of Pb concentration in Bermuda grass and tall fescue in different soil added Pb treatments (control, 50 mg kg^{-1}, 100 mg kg^{-1}). Similar letters show insignificant difference (P<0.05).

	Soil 1		Soil 2	
	Bermuda grass	tall fescue	Bermuda grass	tall fescue
Control	0.26±0.05d	0.65±0.06b	0.33±0.07d	0.29±0.03d
50 mg Pb kg^{-1} Soil	0.79±0.13a	0.57±0.08c	0.71±0.18a	0.57±0.05c
100 mg Pb kg^{-1} Soil	0.73±0.26a	0.65±0.12b	0.8±0.19a	0.66±0.17b

Table 4. Shoot to root ratio of Ni concentration in Bermuda grass and tall fescue in different soil added Ni treatments (control, 50 mg kg^{-1}, 100 mg kg^{-1}). Similar letters show insignificant difference (P<0.05).

	Soil 1		Soil 2	
	Bermuda grass	tall fescue	Bermuda grass	tall fescue
Control	1.68±0.31b	1.3±0.17c	2.55±0.43a	2.06±0.27b
50 mg Ni kg^{-1} soil	1.83±0.14b	0.95±0.22d	1.56±0.27c	0.82±0.1d
100 mg Ni kg^{-1} soil	1.47±0.21c	0.82±0.18d	1.25±0.2c	0.72±0.15d

Tall fescue had also an intensive root system thus it could be an important species for immobilization of the contaminants. Besides, shoot biomass of tall fescue in all treatments was higher than Bermuda grass in all treatments (Fig. 7). Shoot biomass of both plants in all treatments was reduced as the amount of soil metal contamination increased.

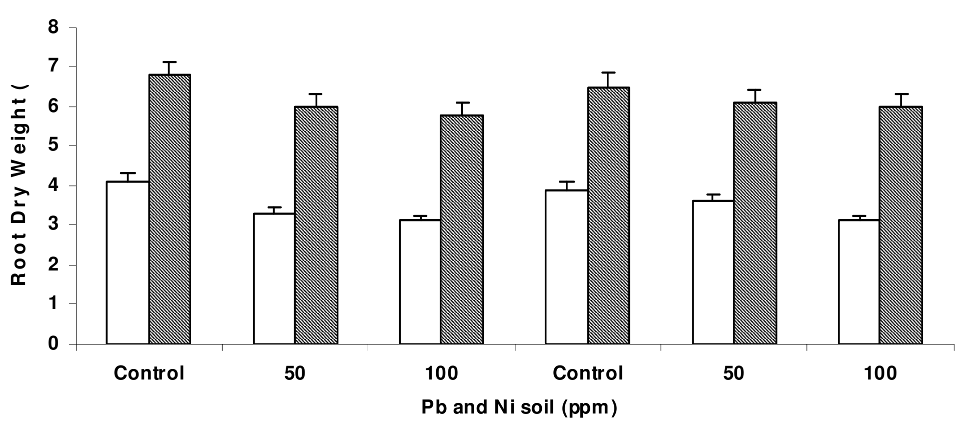

Fig 6. Root biomass of tall fescue (shaded) and Bermuda grass (unshaded) grown in contaminated soils after 10 weeks in different soil added metal treatments (Control, 50 mg kg^{-1}, and 100 mg kg^{-1}). Bars with different letters represent significant difference (P<0.05).

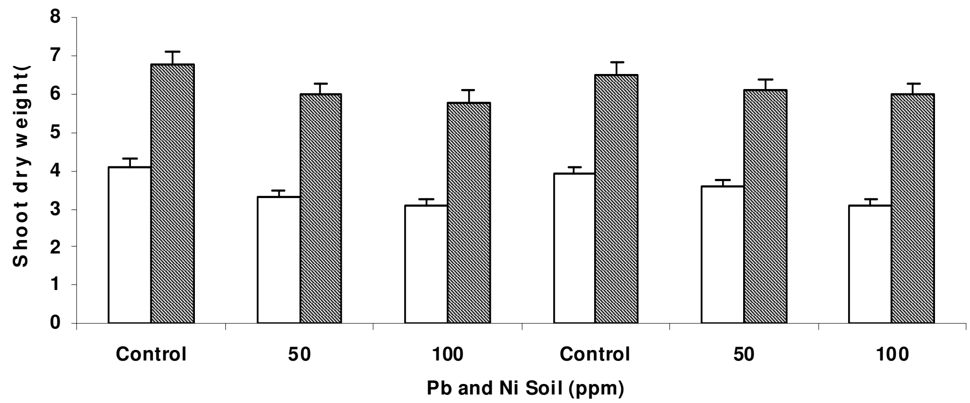

Fig 7. Shoot biomass of tall fescue and Bermuda grass grown in contaminated soils after 10 weeks in different soil added metal treatments (Control, 50 mg kg-1, and 100 mg kg-1). Bars with different letters represent significant difference (P<0.05).

Correlations Between Ni and Pb in Soils, Roots and Shoots

The results showed a significant (P<0.01) correlation (r=0.72) between Ni in roots and shoots (Fig. 8). Correlation coefficient of Pb in roots vs. shoots (r=0.93) and the value for Ni (r=0.72) suggested that translocation from root to shoot was dependent on the gradient of Ni and Pb between roots and shoots (Fig. 9) and could be different in various plant species. There was also a high correlation between DTPA-extractable Ni and Pb in soil and Ni and Pb contents in plants, respectively (Fig 10 and 11). It revealed that Ni and Pb accumulation by tall fescue and Bermuda grass depended on phytoavailability of the metals in soil.

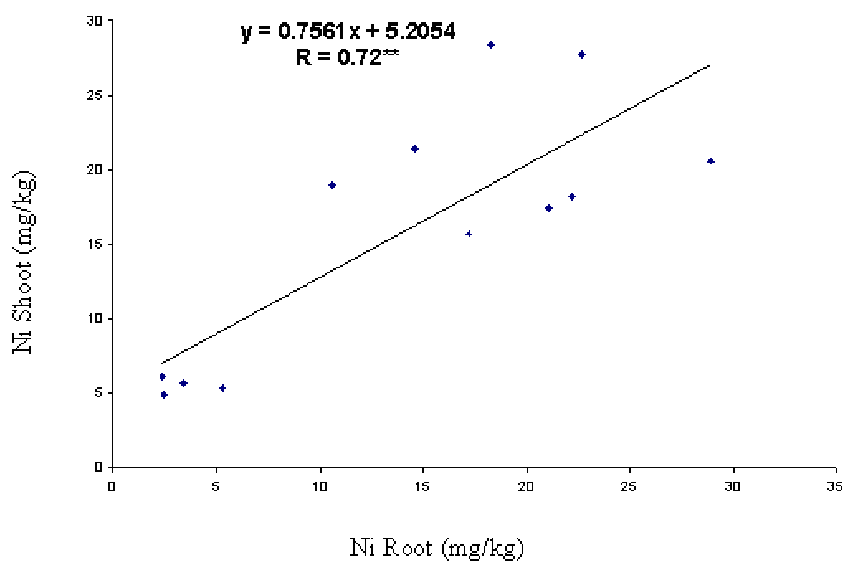

Fig 8. Correlation between Ni content in shoot and root of plants (means significant correlation at P<0.01)**

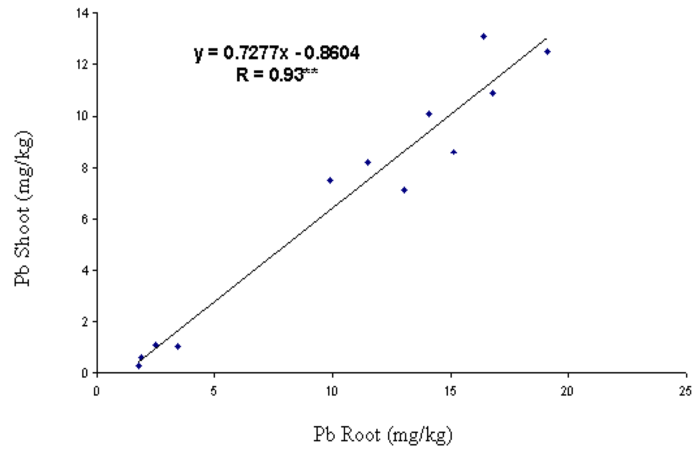

Fig 9. Correlation between Pb content in shoot and root of plants (means significant correlation at P<0.01)**

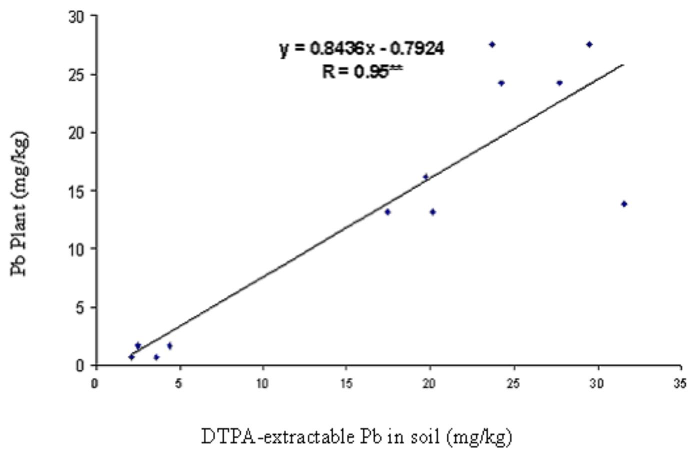

Fig 10. Correlation between DTPA-extractable Pb in soil and Pb accumulated by plants (means significant correlation at P<0.01)**

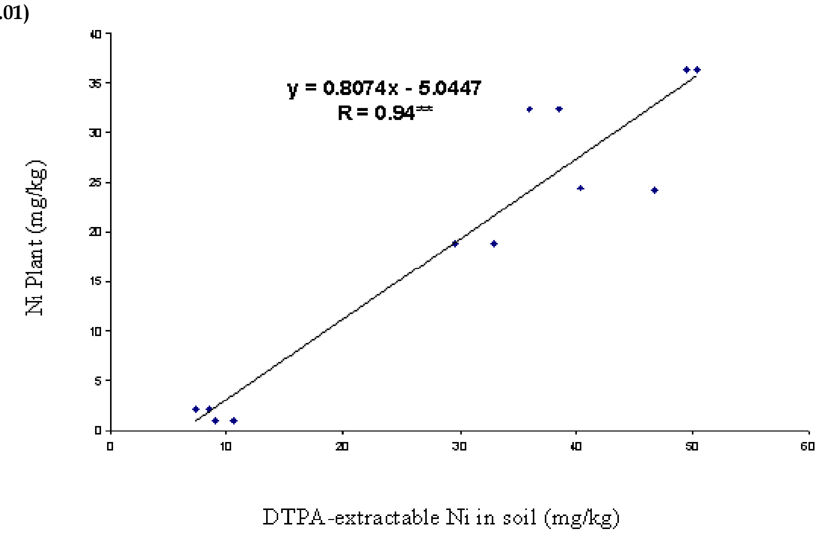

Fig 11. Correlation between DTPA-extractable Ni in soil and Ni accumulated by plants (means significant correlation at P<0.01)**

DISCUSSION

Bioaccumulation of Ni

Knowledge of phytoavailability of Ni in soils is needed to understand the fraction accessible of this element in food chain and plants to evaluate the possibility of using phytoremediation method (Everhart et al., 2006). The higher DTPA-extractable Ni in soil 2 compared to soil 1 caused more accumulation of Ni in roots and shoots of Bermuda grass and tall fescue. The chemical characteristics of soils could affect Ni phytoavailability in soils. More organic matter could increase Ni phytoavailability in soil 2. Ortiz and Alcaniz (2006) reported that phytoavailability of heavy metals increased in soil amended with sewage sludge. Organic matter also had a significant impact on metal sequestration by reducing the solubility of Ni forming complexes with organic functional groups (Nachtegaal and Sparks, 2003). It appeared that the real phytoavailability of many metals depended more on the type of soil organic compounds than on the quantity. Thus, the phytoavailability of many metallic elements increased when they were associated with labile or soluble organic compounds (Alloway and Jackson, 1991). Soil pH is another factor which could change the heavy metals phytoavailability. In this research due to the similarity in both soil's pH, the effect of this factor on DTPA-extractable Ni was negligible. Everhart et al. (2006) reported that higher Ni phytoavailability occured in the soils at pH values of 5.1 and 6.

It seems that Bermuda grass and tall fescue could not transfer Ni from root to shoot easily. Increasing of soil Ni caused a higher accumulation in roots, but no difference was observed in the plant shoots. Plants having ability to accumulate exceeding threshold value of shoot metal content of 0.1% Ni were introduced as hyperaccumulator (Reeves et al., 1995). The maximum amount of Ni accumulated by Bermuda grass and tall fescue was 48-50 mg kg^{-1} dry weight, so these species seem not to be hyperaccumulator during the period of this study. Other reports on Brasicacea family have shown that these plants could accumulate 36 to 48 mg Ni per kg plant dry weight after 6 weeks and suggested that the potential of plants in remediation of Ni-polluted soil could be identified during a long period of plant growth in contaminated soils (Abedi-Koupai, 2003).

Bioaccumulation of Pb

The amounts of accumulated Pb in tall fescue and Bermuda grass were higher in roots compared to the shoots. Root and shoot Pb accumulation increased with increasing the level of soil Pb contents. Because of the higher root biomass of tall fescue than Bermuda grass, this plant could accumulate more Pb in their tissues. It seems that tall fescue had potential to accumulate Pb in root and shoot, but the data did not support the phytoextraction potential of the plant. Begonia et al. (2005) reported that tall fescue may be an efficient Pb-accumulating plant when coupled with other phytoextraction strategies such as lower pH and using of chelates. Higher range of Pb content in soil may accelerate lead accumulation in plant and help finding the potential of tall fescue for phytoremediation. Madyiwa et al., (2002) showed that adding Pb to soil caused increasing Pb phytoavailability and Pb uptake by Star grass. Begonia et al., (2005) indicated that root Pb content increased with increasing level of soil-applied lead. High correlation between DTPA-extractable Pb and accumulated Pb by plants was reported by some researchers (Si et al., 2006; Kim et al., 2003). This means that Pb accumulation by plants depends on Pb phytoavailability in soil. Phytoavailability of heavy metals is also depending on soil characteristics. Jesper and Jensen (1998) indicated that soil pH, texture, cation exchange capacity and soil organic matter might control heavy metal phytoavailability. Lower pH and higher organic matter in soil 2 caused increasing of Pb phytoavailability and bioaccumulation by plants. Si et al. (2006) showed that bioaccumulation of Pb was inversely related to soil pH and clay content. The maximum amounts of Pb accumulated by tall fescue and Bermuda grass were about 30 and 27 mg kg^{-1} dry weight respectively, whereas a hyperaccumulator plant has a potential to accumulate more than 1000 mg Pb per kg dry weight (Reeves et al., 1995). We must also consider that salinity of the soils could have a negative effect on plant growth and therefore decreased bioaccumulation of Pb and Ni by tall fescue and Bermuda grass in this experiment. More research on this subject is recommended.

CONCLUSION

This greenhouse study demonstrated the Pb and Ni bioaccumulation potential of Bermuda grass and tall fescue. Tall fescue showed a higher potential to remove Pb from soil and accumulate it in root, while Bermuda grass could significantly accumulate Ni in shoots. However, these plants did not show the potential of Ni and Pb hyperaccumulation in the soils with moderate metal contents.

ACKNOWLEDGEMENT

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