Assiut J. of Agric. Sci., 42 (Special Issue)(The 5th Conference of Young Scientists Fac. of Agric. Assiut Univ. May,8, 2011) (529-543)

Phytoremediation of Cadmium, Lead and Nickel from the Contaminated Soils by Halophyte Species

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Abstract:

Halophytes are plants that can tolerate and grow on soils having high salt concentration. These plants have been suggested to be more effective in phytoextraction of heavy metals from the contaminated soils compared to the conventional crop plants .This give an offer for a greater potential of phytoremediation research on decontamination of heavy metal polluted soils. Screen house experiment was conducted out to study the accumulation of heavy metals by three halophyte species: *Atriplex amnicola, A. undulate* and *A. lentiformis* .Significant differences were found between the studied species in heavy metals concentration (Cd, Pb and Ni) and transport from the roots to the shoots. *Atriplex lentiformis* could be more effective in the phytextraction of Cd from the contaminated soils.

1. Introduction:

Trace elements such as heavy metals are an important part of the soil ecosystem. However, the accumulation of these elements in the soil or reaching the ground water may be harmful to people, animals, plants and other organisms (Blaylock and Huang, 2000). Geological and anthropogenic activities are sources of heavy metal contamination (Dembitsky, 2003). Sources of anthropogenic metal contamination include industrial effluents, fuel production, mining, smelting processes, military operations, utilization of agricultural chemicals, small-scale industries (including battery production, metal products, metal smelting and cable coating industries), brick kilns and coal combustion (Zhen-Guo *et al.*, 2002). Other sources can include unsafe or excess application of pesticides, fungicides and fertilizers (Zhen-Guo *et al.*, 2002). Additional potential sources of heavy metals include irrigation water contaminated by sewage and industrial effluent leading to contaminated soils and vegetables (Bridge, 2004).Contaminated soils remediation technologies such as soil washing, vitrification and solidification are effective in small areas, they are not only costly but they also cause soil disturbances, and they are not readily accepted by the general public (Martin and Ruby, 2004; Saifullah *et al.*, 2009). Phytoremediation and more

Received on: 2/4/2011 **Accepted for publication on:** 6/4/2011

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specifically phytoextraction, which involves the use of plants to remove metals from the soil into their above-ground biomass which can be harvested using conventional agricultural techniques, has been posed as a cost-effective, environment-friendly alternative restoration strategy for the cleanup of heavy metal contaminated soils (Palmer *et al.* ,2001; Memon and Schröder, 2009; Butcher, 2009). However, the success of phytoextraction depends upon the identification of suitable plant species that tolerate and accumulate heavy metals and produce large amounts of biomass using established agricultural techniques. Plants for phytoextraction, i.e. metal removal from the soil, should have the following characteristics: (i) tolerant to high levels of the metal, (ii) accumulate reasonably high levels of the metal, (iii) rapid growth rate, (iv) produce reasonably high biomass in the field and (v) profuse root system (Baker *et al*, 2000).

Halophytes are plants that can tolerate and grow on soils having high salt concentration and be irrigated with the highly saline water. They show various physiological adaptations that enable them to grow under high salinity conditions. Among the halophyte flora, species belonging to the genus *Atriplex* may be of special interest because of their high biomass production associated with a deep root system able to cope with the poor structure and xeric characteristics of several polluted substrates. These species also naturally produce high amounts of oxalic acid, which may assume positive functions in tolerance mechanisms to heavy metal stress (van Baelen *et al.*, 1980; Mazen and El Maghraby, 1997; Sayer and Gadd, 2001). Fourwing saltbush [*Atriplex canescens* (Pursh) Nutt.)] has been especially recommended for revegetation of mine sites and other harsh environments (Baumgartner *et al.*, 2000; Glenn *et al.*, 2001; Newman and Redente, 2001). *Atriplex* species are able to accumulate high amounts of Se (Vickerman *et al.*, 2002) and some of them have been shown to accumulate B (Watson *et al.*, 1994) or Mo (Voorhees *et al.*, 1991). Halophytes are expected to receive more attention of phytoremediation researchers in the near future. *Atriplex* is one of the important halophyte plants. It is genus contains between 100-200 species, known by the common names of saltbush and orache (or orach). The genus is quite variable and widely distributed including many desert and seashore plants and halophytes. A few numbers of these species were studied for phytoremedation. This study aims to evaluate the accumulation of Cd, Ni and Pb by three species of *Atriplex* (*A. amnicola, A. undulata* and *A. lentiformis*) from a contaminated soil.

2. Materials and methods:

2.1. Soil characterization:

Soil samples were collected from Illwan and Assiut; Egypt where the soils have been irrigated with preliminary sewage waste water for more than 40 years. Part of the collected soil was artificially contaminated with Cd, Ni and Pb in the form of sulphate at a rate of 220 mg kg^{-1} soil for Ni and Pb and 50 mg kg^{-1} soil for Cd and the other part of the soil was left as a control. Particle-size distribution of the soils was performed using the pipette method that was described by Jackson (1970). Soil pH was measured using a digital pH meter in a 1:1 suspension of soil-to-water ratio. Organic matter content of the soil was determined using the dichromate oxidation method described by Wakley and Black (Jackson, 1970). Total carbonates in the soil were estimated gasometrically using a Collins calcimeter and calculated as $CaCO_3$ (Nelson, 1982).The electrical conductivity (EC) was estimated in 1:1 soil to water extract using the salt bridge method (Rhoades, 1982).. DTPA-extractable metal: Cd, Ni and Pb were extracted from the studied soil samples using a 0.005M DTPA (diethylen triamine penta acetic acid) solution buffered at pH 7.3 as described by Lindsay and Norvell (1969). For determination of total heavy metals: The method of Baker and Amacher (1982), which involved the digestion of samples in a mixture of HF-HNO_3-$HClO_4$-H_2SO_4 in Teflon beakers was also employed to extract the total contents of Cd, Ni, and Pb. The physical and chemical characteristics as well as the concentrations of available and total heavy metals of the treated soils under study are presented in Table 1. These soils were classified as a Typic Torripsamments.

2.2. Screen house experiment:

The seeds of studied species were soaked in water for three days with the renewal of water every day. Soaked seeds were placed in pots filled with sand and irrigated as needed. After 30 days the length of seedlings reached to about 15 cm .A pot experiment in a screen house was conducted to study the ability of three species of *Atriplex* (*A. amnicola, A. undulata* and *A.lentiformis*) to accumulate Cd, Ni and Pb. Plastic pots (15 cm in diameter and 20 cm in depth), were filled with 5 kg of the studied soil. Two uniform plants of each species were transplanted in each pot. Pots were carefully watered as needed and fertilized with NPK fertilize mixture (1 g/pot) containing N: P: K at a ratio of 1: 0.4: 0.8

Table 1: Some chemical and physical properties for the studied soil

Property		
Particle size distribution		
Clay %	9.91	
Silt %	22.35	
Sand %	67.74	
Texture	Sandy loam	
$CaCO_3$ %	7.8	
pH (1:1)	7.25	
Organic matter %	2.55	
EC (1:1) dS/m	1.8	
DTPA-extractable metals (mg/kg)	**Control soil**	**Treated soil**
Pb	3.24	30.25
Ni	3.78	83.00
Cd	0.09	12.45
***Total metals** (mg/kg)		
Pb	115	320
Ni	123	320
Cd	1. 05	50

*The permissible limits of the total heavy metals in the soil are 100 mg Pb / kg, 100 mg Ni /kg, (as reported by **Kabata-Pendias and Pendias (1984)** and (0.1–0.5 mg Cd /kg) (as reported by **Scheffer and Schachtschabel (2002)**.

2.3. Plant analysis:

Plants were left in the pots for 90 day after transplanting. At the end of the experiment the plant samples were collected, washed twice with tap water and rinse with distilled water before being separated into shoot and root and oven-dried (70° C) to a constant weight. Dried roots and shoots were ground and submitted to the acid-digestion using a 2:1 HNO_3:$HClO_4$ acid mixture. The digests were analyzed for heavy metals (Ni, Pb and Cd) by atomic absorption spectrophotometer model GBC 906AA. The metal uptake was calculated in order to assess the heavy metal phytoextraction efficiency, the remediation factor (RF) was calculated as the percentage of the element removed by the plant dry above ground biomass from the total metal content in the soil (Neugschwandtner *et al.*, 2008). The translocation factor (TF), which evaluates the capacity of a plant to transfer metals from roots to shoots, was also computed as the ratio of the metal concentration in the shoots to that in the roots.

2.4. Statistical Analysis:

The experimental design of this experiment was Randomized Complete Blocks Design (RCBD) with three replicates. The means were compared using the least significant difference (LSD) values at a 5% level of significance. The collected data were statically analyzed using MSTAT computer program as described by Freed *et al.* (1987)

3. Results and Discussion:

The dry matter production of root and shoot was shown in table 2. The roots weight of the studied plants ranged between 6.52 and 7.85 with an average of 7.22 gm in the treated soil, while it ranged between 5.93 and 6.48 with an average of 6.27 gm in the control soil. The data also indicated that the shoots weight of the studied plants ranged between 57.78 and 62.34 with an average of 60.72 gm in the treated soil, while it ranged between 53.14 and 60.55 with an average of 57.11 gm in the control soil.

Table 2: Root and shoot weight of *Atriplex* species (g / pot)

Species	Root		Shoot	
	Treated soil	Control soil	Treated soil	Control soil
A.. amnicola	6.52	6.42	57.78	53.14
A.. undulata	7.30	6.48	62.34	60.55
A.. lentformis	7.85	5.93	62.04	57.64
Mean	7.22	6.27	60.72	57.11
LSD s $_{0.05}$	ns		ns	
LSD t $_{0.05}$	ns		ns	
LSD st $_{0.05}$	ns		ns	

Treated soil was spiked with 220 mg kg^{-1} soil for Ni and Pb and 50 mg kg^{-1} soil for Cd.

(LSD) Least Significant Difference values at a 5% level of significance for the species (s), the treatment (t) and the interaction of species and treatment (st).

No significant differences were found between the species in dry mater production but the *A. amnicola* gave the lowest values for root and shoot weight. *.Atriplex* species grown on the treated soils did not affect by the high concentration of heavy metals because its high toleranance to heavy metals. Similar results were obtained by Lutts *et al.* (2004); Kadukova *et al.* (2004); Sai Kachou *et al.* (2009).

Table 3: Concentrations of Cd, Pb and Ni in roots and shoots of Atriplex species (ppm)

Treatment	SPECIES	Cd		Pb		Ni	
		Root	Shoot	Root	Shoot	Root	Shoot
Treated soil	*A. amnicola*	34.37	12.83	71.00	25.23	51.43	23.77
	A. undulata	160.67	71.67	321.13	168.97	55.37	30.80
	A .lentformis	269.33	141.10	614.50	336.50	58.97	29.60
Mean		154.79	75.20	335.54	176.90	55.26	28.06
Control soil	*A. amnicola*	0.22	0.07	14.78	7.15	14.59	5.84
	A. undulata	0.24	0.04	14.98	8.48	17.54	6.70
	A .lentformis	0.19	0.07	16.93	5.50	14.41	6.97
Mean		0.22	0.06	15.56	7.04	15.51	6.50
LSD s $_{0.05}$		17.91	9.08	54.06	14.77	ns	3.10
LSD t $_{0.05}$		14.62	7.41	44.14	12.06	4.63	2.53
LSD st $_{0.05}$		25.32	12.84	76.45	20.89	ns	ns

(LSD) Least Significant Difference values at a 5% level of significance for the species (s), the treatment (t) and the interaction of species and treatment (st).

Treating the soil with the heavy metals had a significant effect on the concentration of Cd, Pb and Ni in the roots and shoots of the halophyte species as shown in table 3. Most of the taken elements by the studied species were found to be with the roots. Cadmium concentrations in the roots of studied species ranged between 34.37 and 269.33 ppm with an average of 154.79 ppm for the treated soil, while it ranged between 0.19 and 0.24 with an average of 0.22 ppm in the roots of the plants germinated in the control soil. Cadmium concentrations in the shoots of studied species ranged between 12.83 and 141.10 with an average of 75.20 ppm for the treated soil, while it ranged between 0.04 and 0.07 with an average of 0.06 ppm for the control soil.

Table 4: The ratio between the metal stored in the shoot to the metal absorbed by the whole plant (%)

SPECIES	Cd		Pb		Ni	
	Treated soil	Control soil	Treated soil	Control soil	Treated soil	Control soil
A. amnicola	27.02	26.45	26.10	32.58	31.68	28.52
A. undulata	31.13	15.05	34.35	36.11	35.76	27.14
A. lentformis	34.29	26.90	35.66	24.47	33.44	32.89
Mean	30.81	22.80	32.04	31.07	33.63	29.52
LSD s $_{0.05}$	ns		3.43		ns	
LSD t $_{0.05}$	6.76		ns		3.84	
LSD st $_{0.05}$	ns		4.85		ns	

(LSD) Least Significant Difference values at a 5% level of significance for the species (s), the treatment (t) and the interaction of species and treatment (st).

The ratio between the Cd stored in the shoot to the Cd absorbed by the whole plant varied between 27.02 and 34.29 with an average of 30.81 % for the treated soil, while in it ranged between 15.05 and 26.45 with an average of 22.80 % in the control soil as shown in table 4. The highest value of Cd concentration in the shoot was found in the *A. lentifomis* (141 ppm) in the treated soil as shown in table 3. The plant uptake of Cd by many species of *Atriplex* was reported. Lutts *et al.* (2004) studied the accumulation of Cd by the halophyte species Mediterranean saltbush (*Atriplex halimus* L) and they found that mean Cd accumulation in aerial parts was 440 mg / kg, but the roots stored more Cd (3000 ppm). High Cd concentration in the root compared to the shoot of *Atriplex* plants was found also by Nedjimia and Daoud (2009) and Ghnaya *et al.* (2005, 2007).

Lead concentrations in the plants grown on the control soil were very low comparing to the plants grown on treated soil as shown in table 3. Lead concentrations in the roots of studied species as a % of the metal absorbed lead ranged between 71.00 and 614 with an average of 335.54 ppm for the treated soil, while it ranged between 14.78 and 16.93 with an average of 15.56 ppm for the control soil. Lead concentrations in the shoots of studied species ranged between 25.23 and 336.50 with an average of 176.90 ppm for the treated soil, while it ranged between 5.50 and 7.15 with an average of 7.04 ppm for the control soil. The ratio between the Pb stored in the shoot to the Pb absorbed by the whole plant varied between 26.10 and 35.66 with an average of 32.04 % for the treated soil, while it ranged between 24.47 and 36.11 with an average of 31.07 % in the control soil as shown in table 4. The highest value of Pb concentration was found in the root of *A. lentifomis* (614ppm) in the treated soil. The highest value of Pb concentration was found in the shoots of *A. lentiformis* (336 ppm) in the treated soil. Toxicity level of Pb in leaves was reported by Orcutt and Nilsen (2000) to range from 30-300 ppm. The ratio of Pb stored in the shoot to the Pb absorbed by the whole plant varied between 24 to 36 % as shown in table 4 .Kadukova et al (2004) found that the Pb stored in the root of *Atriplex halmus* was 93-98 % from the total Pb absorbed by plant. Wozny (1995) reported that the roots can take up lead 3-50 times more than the leaves.

Treating the soil with the heavy metals increased the plant uptake of Ni for the studied species while there were no significant differences between the species in concentration of Ni in root and shoot as shown in table 3.

Nickel concentrations in the roots compared with the total Ni absorbed of studied species ranged between 51.43 and 58.97 with an average of 55.26 ppm for the treated soil, while it ranged between 14.41 and 17.54 with an average of 15.51 ppm for the control soil. Nickel concentrations in the shoots of studied species compared with the total Ni absorbed ranged between 23.77 and 30.80 with an average of 28.06 ppm for the treated soil, while it ranged between 5.84 and 6.97 with an average of 6.50 ppm for the control soil. The ratio between the Ni stored in the shoot to the Ni absorbed by the whole plant varied between 31.68 and 35.76 with an average of 33.63 % for the treated soil, while in it ranged between 27.14 and 32.89 with an average of 29.52 % in the control soil as shown in table 4. The studied species of *Atriplex* had low ability to absorb and accumulate Ni from contaminated soils. Most of the accumulated Ni was stored in the root. Similar results were reported by Sai Kachou *et al.*, 2009. They found that certain *Atriplex* species had low ability to translocate Ni to the shoots. The criterion for defining Ni hyperaccumulation in plants is 1000 mg Ni / Kg of dry mater (Reeves, 1992; Baker *et a l.*, 2000).

The translocation factor (**TF**) as shown in Table (5) is defined as the ratio of the metal concentration in the shoots to those in the roots of a plant. It is used to evaluate the capacity of a plant to transfer a metal from the roots to the shoots (Zhao *et al.*, 2003).

Table 5: Elemental translocation Factor (TF) for the different species

SPECIES	Cd		Pb		Ni	
	Treated soil	Control soil	Treated soil	Control soil	Treated soil	Control soil
A .amnicola	0.37	0.40	0.35	0.48	0.47	0.40
A. undulata	0.46	0.18	0.52	0.57	0.56	0.38
A .lentformis	0.52	0.37	0.56	0.33	0.50	0.50
Mean	0.45	0.32	0.48	0.46	0.51	0.42
LSD s $_{0.05}$	ns		0.06		ns	
LSD t $_{0.05}$	ns		ns		0.08	
LSD st $_{0.05}$	ns		0.11		ns	

(LSD) Least Significant Difference values at a 5% level of significance for the species (s), the treatment (t) and the interaction of species and treatment (st).

The **TF** values of the studied plants ranged between 0.37 and 0.52 with an average of 0.45 for Cd in the treated soil, while it ranged between 0.18 and 0.40 with an average of 0.32 in the control soil. In case of Pb the **TF** values of the studied plants ranged between 0.35 and 0.56 with an average of 0.48 in the treated soil, while it ranged between 0.33 and 0.57 with an average of 0.46 in the control soil. Nickel translocation factor values were ranged between 0.50 and 0.56 with an average of 0.51 for Ni in the treated soil, while it ranged between 0.38 and 0.50 with an average of 0.42 in the control soil. Significant differences between plant species were found only in the case of Pb. In the treated soil *A. amnicola* had the lowest TF in Cd and Pb where the *A. lentformis* and *A. undulate* plants had a high TF in Cd and Pb. All the TF values were less than one as shown in table 5 .The studied plants absorbed high amount of Cd and Pb but their ability to translocate them to the shoot was low. These results were similar to that obtained by (Kadukova *et al.*, 2004; Lutts *et al.*, 2004, Nedjimia and Daoud, 2009; Sai Kachou *et al.*, 2009; Ghnaya *et al.*, 2005, 2007).

The success of phytoextraction process depends upon both shoots biomass and shoots metal concentration. The remediation factor (**RF**) was calculated to asses the ability of plants in phytoextraction. This parameter shows the real ability of a plant in remediation of contaminated soils. In our study data of this parameter were presented in table 6. The remediation factor for the studied plants were ranged between 030 and 3.49 with an average of 1.86 for Cd in the treated soil, while it ranged between 0.50 and 0.77 with an average of 0.65 in the control soil. In the case of Pb the **RF** values of the studied plants were ranged between 0.10 and 1.31 with an average of 0.62 in the treated soil, while it ranged between 0.55 and 0.89 with an average of 0.70 in the control soil. In case of nickel; remediation factor values were ranged between 0.09 and 0.12 with an average of 0.11 for Ni in the treated soil, while it ranged between 0.50 and 0.66 with an average of 0.60 in the control soil as shown in table 6.

Table 6: % Remediation Factor (RF) for the different species

SPECIES	Cd		Pb		Ni	
	Treated soil	Control soil	Treated soil	Control soil	Treated soil	Control soil
A. amnicola	0.30	0.69	0.10	0.67	0.09	0.50
A. undulata	1.79	0.50	0.45	0.89	0.12	0.66
A. lentformis	3.49	0.77	1.31	0.55	0.12	0.64
Mean	1.86	0.65	0.62	0.70	0.11	0.60
LSD s $_{0.05}$	0.21		0.16		ns	
LSD t $_{0.05}$	0.18		ns		0.08	
LSD st $_{0.05}$	0.30		0.23		ns	

(LSD) Least Significant Difference values at a 5% level of significance for the species (s), the treatment (t) and the interaction of species and treatment (st).

High significant differences were found between the species in the **RF** of Cd and Pb. **RF** values for Cd was high compared with Ni and Pb. Plants of *A. lentiformis* had the highest **RF** value for Cd (3.49) and Pb (1.31) followed by *A. undulata*.

From the phytoremediation perspective a good metal accumulator should meet the following criteria: (i) it should be able to accumulate high level of the metal concerned in its harvestable tissues, (ii) it should be a fast growing species, and (iii) it should possess well developed root system **(Qian et al., 1999).**

The accumulation strategy caused high uptake of metal and storage in vacuoles to prevent metal toxicity. The extreme level of metal tolerance in vascular plants is called hyperaccumulation. Hyperaccumulators are defined as higher plant species whose shoots contain > 100 mg Cd / kg kg dry wight , > 1000 mg Ni, Pb, and Cu / kg dry weight, > 10 000 mg Zn and Mn / kg dry weight when grown in metal-rich soils **(Baker *et al.*, 2000).** The strategies of resistance in those plants involve several mechanisms such as the vacuolar sequestration of heavy metals linked to overproduced organic acids (malate, citrate, or oxalate) or phytochelations produced from glutathione (Salt *et al.*, 1998).

In this study the tested plants tolerate levels of Cd, Pb, and Ni in the soil and *A. lentiformis* tolerate the high concentration of Cd and Pb in leaves. *A. lentiormis* plants were able to remove 3.49 % of the total Cd from the soil through the experimental period (three months)

4. Conclusion:

Atriplex plants, have an excellent tolerance to drought and salinity. These plants could be used to remediate contaminated soils with heavy metals .This study indicated that *Atriplex* plants accumulated metal in roots and translocated few of these elements in the harvestable parts of the plant. Significant differences in heavy metals (Cd, Pb and Ni) concentration and transport from the roots to shoots were found between the three species of *Atriplex*.The data obtained in this study suggest that Atriplex lentiformis may be more effective in the phytextraction of Cd from the contaminated soils. Therefore, the above mentioned results indicate that *Atriplex* may be used to remediate the polluted soils with heavy metals. More studies are needed in this field about another sources of heavy metals pollution under Upper Egypt conditions.

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Assiut J. of Agric. Sci., 42 (Special Issue)(The 5th Conference of Young Scientists Fac. of Agric. Assiut Univ. May,8, 2011) (529-543)

والنيكل بواسطة أنواع من النباتات المحبة للملحية

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تنمو النباتات المحبة للملحية فى الاراضى الملحية مقاومة التركيز المرتفع للاملاح فى التربة .هذه النباتات اقترحت ان تكون اكثر فاعلية فى الاستخلاص النباتى للعناصر الثقيلة من الأراضى الملوثة مقارنة بنباتات المحاصيل التقليدية مما أعطى فرصة أكبر للبحث فى مجال العلاج النباتى للأراضى الملوثة بالعناصر الثقيلة.أجريت تجربة فى الصوبة السلكية لدراسة تراكم العناصر الثقيلة بواسطة ثلاث أنواع من النبتات المحبة للملحية هى *Atriplex amnicola, A. undulate, A. lentiformis* . و قد وجدت اختلافات معنوية فى تركيزات العناصر الثقيلة فى الأنواع المدروسة كما اختلفت قدرة هذه النباتات فى نقل العناصر الثقيلة من الجذر الى الساق. و قد وجد ان نوع *A. lentiformis* أكثر فاعلية فى الاستخلاص النباتى للكادميوم من الاراضى الملوثة. وبذلك فانه يقترح استخدامه فى المعالجة الحيوية للتربة الملوثة بعنصر الكادميوم.