Halophytic Plants for Phytoremediation of Heavy Metals Contaminated Soil

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Abstract: Using of halophyte species for heavy metal remediation is of particular interest since these plants are naturally present in soils characterized by excess of toxic ions, mainly sodium and chloride. In a pot experiment, three halophyte species *viz. Sporobolus virginicus*, *Spartina patens* (monocotyledons) and *Atriplex nammularia* (dicotyledon) were grown under two levels of heavy metals: 0 level and combinations of 25 mg Zn + 25 mg Cu + 25 mg Ni/kg soil. The three species demonstrated high tolerance to heavy metal salts in terms of dry matter production. *Sporobolus virginicus* reduced Zn, Cu, and Ni from soil to reach a level not significantly different from that of the untreated control soil. Similarly, *Spartina patens* significantly reduced levels of Zn and Cu but not Ni. *Atriplex nummularia* failed to reduced Zn, Cu and Ni during the experimental period (two months). Only *Sporobolus virginicus* succeeded to translocate Zn and Cu from soil to the aerial parts of the plant. The accumulation efficiency of Zn and Cu in aerial parts of *Sporobolus virginicus* was three and two folds higher than *Spartina patens* and around six and three times more than *Atriplex nammularia* for both metals, respectively.

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1. Introduction:

The contamination of soil by heavy metals is one of the most serious environmental problems and has significant implications for human health. Some industrial activities and agricultural practices increase their level in the substrate, and the possible introduction of the elements in the food chain is an increasing human health concern (**Cakmak *et al.*, 2000).** Engineering industrial techniques may efficiency be used to clean up contaminated soils but most of them require sophisticated technology and are therefore expensive and suitable only for small polluted areas **(Lutts *et al.*, 2004).** Furthermore, these technologies are not only costly, but they also cause soil disturbances and they are not readily accepted by the general public **(Gardea-Torresdey *et al.*, 2004 and Manousaki *et al.*, 2007).** Phytoremediation has been highlighted as an alternative technique to traditional methodologies, for the removal of heavy metals from soil. Two approaches have been generally proposed for the phytoremediation of heavy metals. The first one is using of natural hyperaccumulator plants with exceptional metal-accumulating capacity. However, the hyperaccumulator plants usually accumulate only a specific element, tended to grow slowly and to have a low biomass. Many of them are not suitable to be used as phytoextraction in the field. While the second is utilizing of high-biomass plants whereas, relatively high amounts of the metal accumulated by plants is often translocated from the root to the more easily harvestable shoots **(Chen *et al.*, 2004 and Manousaki *et al.*, 2007).** Disposal options include pyrolysis, composting, and compaction as pretreatment steps in order to reduce the volume of plant material and incineration, ashing, liquid extraction, or direct disposal-since plants are easier to dispose of than soil as final disposal.

Zinc, copper and nickel are of significant importance because their excessive amounts in the soil can arise not only due to human economic activity but also because of nature soil forming processes. In fact, these heavy metals occur in some parent material of soils in high concentrations. Thus, Cu content in soil was shown to vary 1000-fold (from 2 to 2000 mg/kg soil); Zn more than 4000 fold (from 25 to more than 10000 mg/kg soil **(Kholodova *et al.*, 2005).** Therefore, searching for plants capable of accumulation the high concentrations of these metals in their shoots is of interest in respect to phytoremediation technology. Growing of such plants permit natural soil cleaning up from excess heavy metals, thus helping other, less tolerant plant species to inhabit the region and consequently soil can be restored for farming **(Lombi *et al.*, 2001; Gleba *et al.*, 1999; Lasat, 2002 and Zhulidov *et al.*, 2002).**

Halophytes are the plants naturally grow in high salt affected soils. They have developed different strategies to survive and complete their life cycles, sometimes in very high salinity level reached over seawater salinity level.

Sporobolus virginicus, *Spartina patens* and *Atrriplex nummularia* are halophyte species remove excess salt from the soil by various strategies. The three plants have salt glands through which salt is excreted from their leaves. This capability to uptake salt from soil and translocated from root into aboveground shoot without, in most cases, a significant decrease of shoot biomass production is an advantage not available in conventional crops. Moreover, halophytes are found usually in arid and semi arid regions and can grow on land of marginal quality which allows their use for phytoremediation of soils with low fertility and poor soil structure, resulting in low operating costs.

The aim of this study was to investigate the possibility of using some halophytic plants as phytoremediation as well as its efficiency under Egyptian conditions.

2. Materials and Methods

To investigate the possibility of using some halophytic plants as phytoremediation a pot experiment was carried out in greenhouse at the Faculty of Agriculture, Ain Shams University, Cairo, Egypt. *Sporobolus virginicus* (L.) Kunth (smyrna) and *Spartina patents* (Aiton) Muhl. were introduced from Delaware, U.S.A. Rhizomes of these grasses were first transplanted in pots under greenhouse at the National Research center Cairo, Egypt. Some pots of these grasses were again transplanted into greenhouse of Agricultural Botany Dept. at Faculty of Agriculture, Ain Shams University. Rhizome cuttings 5-8 cm in length were rooted in tap water. *Atriplex nummularia* Lindl seedling was obtained from Desert Research Center, Cairo, Egypt and planted in the greenhouse of Agricultural Botany Department on October 2006. Two years later, stem cuttings 30 cm length were taken and rooted in tap water. All rooted cuttings were planted in pots on September 1st 2008.

Factorial experiment was conducted in randomized complete block design including 3 halophytic species and 2 levels of heavy metals (without and with 25 mg Zn + 25 mg Cu + 25 mg Ni/kg soil) with 5 replicates. The combination of metals was based on Zn equivalent of 260 mg/kg (the level of Zn caused toxic effect for most plants) in which Zn was replaced equally with Cu or Ni assuming that Cu was twice as toxic and Ni was eight times as toxic as Zn **(Davies, 1980)**. So that the amounts of Zn, Cu and Ni used were 275 mg/kg as Zn equivalent. Soil sample was characterized by pH = 7.8, ECe = 2.5 dS/m, $CaCO_3$ = 3.2 %, organic matter = 0.02 %, Clay = 2 %, Silt = 3 % and Sand = 95 %. The soil texture grade was sand (Typic Torripsament). Each pot was filled with 5 kg sandy soil. Soil was well mixed with 1.88g ammonium sulfate (equal 190 kg N/ha), 1.39g potassium phosphate (equal 190kg K and 150kg P/ha). Pots of treatment with heavy metal were well mixed with 0.548g $ZnSO_4.7H_2O$ + 0.265g $CuCl_2$ + 0.506g $NiCl_2.6H_2O$. Pots were watered to keep moisture content approximately at 60% of water holding capacity. Eight weeks after planting date the plants were harvested and soil samples were taken from each pot, then plant and soil samples were air dried and kept in plastic bags for analysis.

All soil chemical properties were determined according to **Page *et al.* (1982)**. Soil pH was determined in 1:2.5 soil, water suspension. Calcium carbonate was determined with calcimeter. Available Zn, Cu and Ni were extracted by DTPA extractant and then determined using atomic absorption spectrophotometer (Varian Spetra AA20, Victoria, Australia).

Dried plant samples were wet ashed with the ternary acid mixture, HNO_3, $HClO_4$, H_2SO_4. Plant contents of Zn, Cu and Ni were determined as mentioned above.

All parameters of soils and plants were analyzed statically by multiple factor analysis of variance in randomized complete block design using Tukey's multiple range test of significance at 5% level as described by **Steel and Torrie (1980)**.

3. Results

Data presented in Table (1) and illustrated in Fig. (1) showed that the heavy metal salts had no significant effect on aboveground dry matter production of the three halophyte species used in this study. On the other hand, *Sporobolus virginicus* recorded the highest significant value of areal biomass followed by *Spartina patens*. While *Atriplex nummularia* produced the least significant value of shoot yield during the experimental period.

The total removal of Zn, Cu and Ni from the contaminated soil by plants is presented in Table (1) and Figs. (2-4). Data indicated that *Sporobolus* plants remediate soluble Zn, Cu and Ni salts from treated soil causing significant reduction of the metals level to be statistically equal with untreated control soil. However, *Sporobolus* plants reduced level of soluble Ni in contaminated soil yet less than that detected for Zn and Cu. *Spartina patens* came descendingly after *Sporobolus virginicus* and significantly reduced level of Zn and Cu in contaminated soil to be similar with untreated soil. In spite of obviously reduction of soluble Ni in treated soil by *Spartina* plants, but Ni level still significant higher than that found in untreated soil. *Atriplex nummularia* failed to achieve the same efficiency of the other two plants. The level of Zn, Cu and Ni in contaminated soil exhibited the highest significant values by using *Atriplex nummularia* compared with either *Sporobolus* or *Spartina* plants.

Almost the same pattern was found by examining the heavy metals concentration and uptake by the plant shoots as presented in Figs (5-10). Here again, the highest accumulation levels of Zn, Cu and Ni were achieved by *Sporobolus virginicus* to record 48.4, 8.7 and 28.3 $g.Kg^{-1}dw$, followed by *Spartina patens* (16.4, 4.4 and 28.7 $g.Kg^{-1}dw$). Meanwhile, *Atriplex nummularia* accumulated the lowest values 8.5, 2.5 and 23.0 $g.g^{-1}dw$ for Zn, Cu and Ni, respectively. However, only Zn and Cu achieved a higher significant accumulation level by *Sporobolus* plants above all other treatments. No significant differences were detected either for *Spartina* or *Atriplex* plants on concentration or uptake of heavy metals in their aerial part above untreated ones.

Table 1. Analysis of variance and multiple range test of Zn, Cu and Ni available in soil, concentrations and uptake by different plants untreated and treated with heavy metals.

Source of variance		Dry weight	Soil available			Plant content			Plant uptake		
			Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni
Analysis of variance											
Type of plant		***	***	***	***	***	*	NS	***	***	***
Heavy metals		NS	***	***	***	***	**	**	***	***	**
Interactions of plants X metals		NS	***	***	**	***	**	NS	***	***	NS
Tukey's Multiple range test											
Main effect											
Type of plant	*Spartina*	B	B	B	B	B	A	NS	B	B	B
	Sporobolus	A	B	B	B	A	A	NS	A	A	A
	Atriplex	C	A	A	A	B	A	NS	B	B	B
Heavy metals	without	NS	B	B	B	B	B	B	B	B	B
	With	NS	A	A	A	A	A	A	A	A	A
Interactions											
Spartina Without heavy metals		NS	B	B	C	B	B	NS	BC	B	NS
Spartina with heavy metals		NS	B	B	B	B	B	NS	BC	B	NS
Sporobolus Without heavy metals		NS	B	B	C	B	B	NS	B	B	NS
Sporobolus with heavy metals		NS	B	B	BC	A	A	NS	A	A	NS
Atriplex Without heavy metals		NS	B	B	BC	B	B	NS	C	B	NS
Atriplex With heavy metals		NS	A	A	A	B	B	NS	C	B	NS

*,** and *** significant effect at $P < 0.05$, 0.01 and 0.001, respectively, NS = not significant.
The same streaks within factors are not different but a value A > B > C …etc at 5% level.

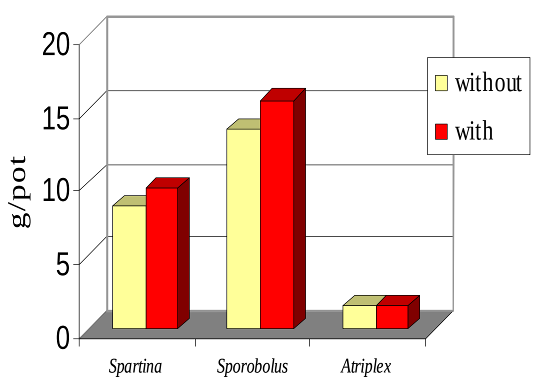

Fig. 1. Effect of heavy metals treatment on the dry weights of different halophyte plants.

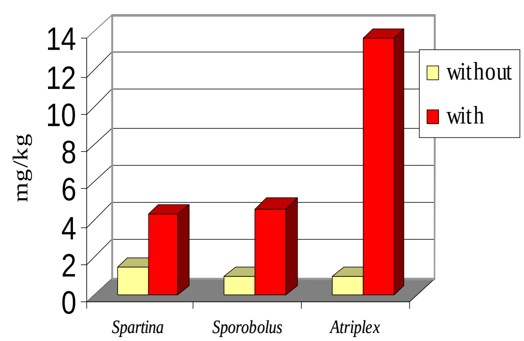

Fig. 2. Effect of different plants on the available Zn in the untreated and treated soil with heavy metals.

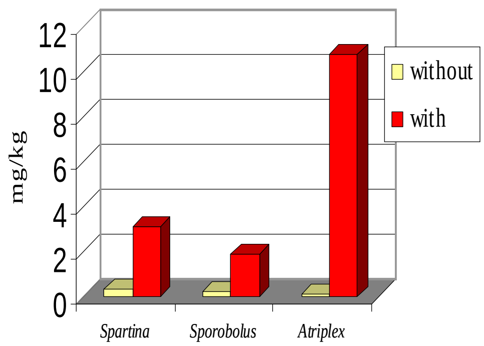

Fig. 3. Effect of different plants on the available Cu in the untreated and treated soil with heavy metals.

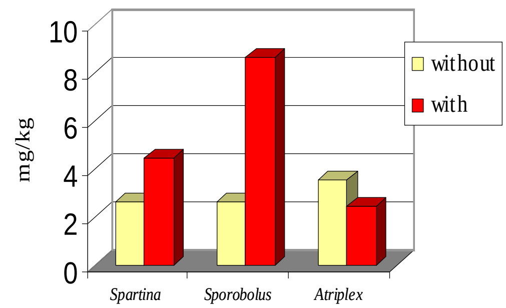

Fig. 6. Copper concentration in different plants grown on untreated and treated soil with heavy metals.

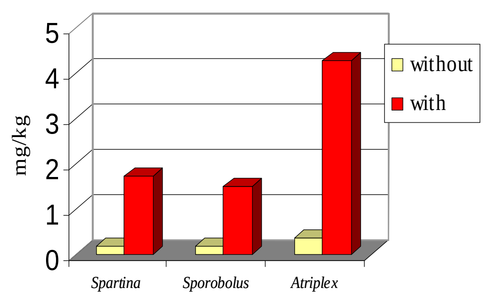

Fig. 4. Effect of different plants on the available Ni in the untreated and treated soil with heavy metals.

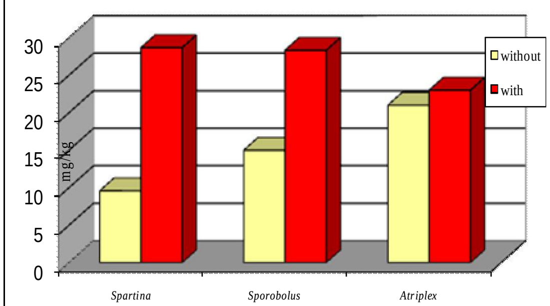

Fig.7. Nickel concentration in different plants grown on untreated and treated soil with heavy metals.

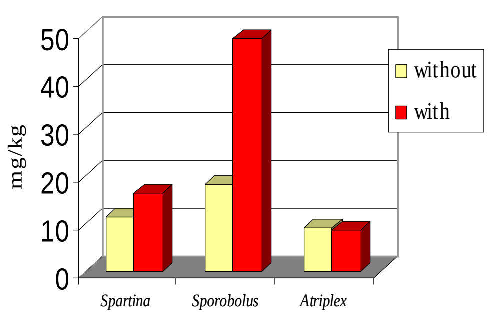

Fig. 5. Zinc concentration in different plants grown on untreated and treated soil with heavy metals.

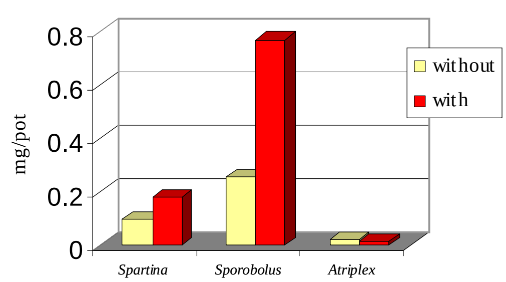

Fig. 8. Zinc uptake by different plants grown on untreated and treated soil with heavy metals.

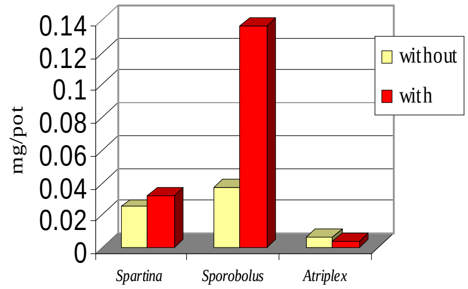

Fig. 9. Copper uptake by different plants grown on untreated and treated soil with heavy metals.

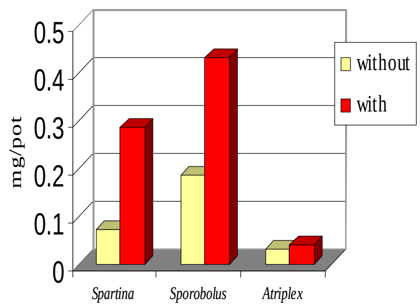

Fig. 10. Nickel uptake by different plants grown on untreated and treated soil with heavy metals.

4. Discussion

The present work showed that the halophytic species *Sporobolus virginicus*, *Spartina patens* (monocotyledons) and *Atriplex nummularia* (dicotyledon) demonstrated a high tolerance to Zn, Cu and Ni salts. Also they accumulated these elements without showing any significant decrease in terms of dry biomass production. On the other hand, there were significant differences in the aboveground biomass production among the three species, the highest significant biomass values were found in *Sporobolus virginicus* followed by *Spartina pa*tens but *Atriplex nummularia* recorded the lowest value during the experimental period. In this respect, **Kholodova *et al.* (2005)** reported that seeds and young seedlings of the halophyte *Mesembryanthemum crystallinum* demonstrated a high tolerance of heavy metals salts. They added that, the halophyte *Mesembryanthemum crystallinum* could grow at $CuSO_4$ concentrations 200-fold and $ZnSO_4$ concentrations 800-fold higher than those in standard nutrient medium providing for normal development. On the other hand, the use of halophyte species for heavy metal remediation is of particular interest because these plants are naturally present in environments characterized by an excess of toxic ions, mainly sodium and chloride. Several studies demonstrated that some tolerance mechanisms operating at the whole plant level are not always specific to sodium but also for other toxic elements such as copper, zinc or cadmium **(Hagemeyer and Waisel, 1988; Neumann *et al.*, 1995; McFarlane and Burchett, 1999; Lutts *et al.*, 2004; Reboreda and Cacador, 2007 and Manousaki *et al.*, 2007).** Drought and/or salinity resistance mechanisms in halophyte species may indirectly contribute to heavy metals tolerance, since high level of heavy metals salts are responsible for secondary water stress in plant **(Poschenrieder *et al.*, 1989)**.

Heavy metals stress also had a limited effect on the concentration of other elements. A decrease in calcium may be partially explained by the fact that divalent cations can translocate in the plant through nonselective calcium channels**.** Also, a strong inhibition of photosynthesis may result from iron deficiency in Cd-treated plants or iron transportation from root to shoot was reduced under zinc-treated plants **(Lutts *et al.*, 2004).**

On the other hand, the three halophytic plants used in the present study have salt glands. In this respect, **Freitas and Breckle (1993)** reported that many halophyte species are able not long to accumulate high amount of Na in trichomes covering the leaf surface but also other elements may also accumulate when present in excess. Trichomes accumulate both Zn and Cd in the resistant species *Arabidopsis halleri* (L.) **(Küpper *et al.*, 2000)**, and Cd in the halophyte plant *Tamarix aphylla* (L.) **(Hagemeyer and Waisel, 1988)**. Specific over expressions of a gene coding for a metallothionein (MTZ) has been reported in trichomes of various halophyte species **(Folcy and Singh, 1994 and Garcia-Hernandez *et al.*, 1998),** there were constituted important sites for toxic metal accumulation **(Lutts *et al.*, 2004)**. In the present study, the three halophytes used have the ability to excrete the excess NaCl under salt effect through salt glands and trichomes covering their leaf surface. Therefore, it may be suggested that in Cu and Ni salts were excreted through trichomes and salt glands to leaf surface then washed out mainly by dew. This gave the reason for sharply reduction of heavy metals level from the contaminated soil without significant accumulation in plant shoots in particularly for *Spartina* and also for Ni concentration in both *Sporobolus* and *Spartina* plants. Also, gave the answer why these plants are tolerant for the high concentration of heavy metals. On the other hand, the three halophytes are C_4 plants characteristic by fast growing and high biomass in short time than other conventional crop particularly without fertilizer supply. In this respect **Chen *et al.* (2004)** mentioned that plants used for phytoextraction should be tolerant to the high concentrations of metals without significant inhibitions of growth or reduction in biomass production.

In light of recently work it appears that plants used for phytoextraction must first become well developed and established in contaminated soil.

Sporobolus and *Spartina* plants responded faster by transplanting and grown better than *Atriplex*. That might be due to the type of root system, whereas the monocytedonous *Sporobolus* and *Spartina* plants characterized by adventurous root system which grown faster after transplanting than tap root of *Atriplex*. *Sporobolus* reached its maximum aboveground biomass after two months. Meanwhile, the same period was not enough for *Atriplex* plant to reach its maximum aerial biomass. The aim of phytoremediation is to reduce the level of metals in the contaminated soil to acceptable levels within a reasonable time frame. In light of recently work, it appears that *Sporobolus* plant has the greatest efficiency for phytoremediation of Zn, Cu and Ni salts from contaminated soils.

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