Phytoremediation of Soil Heavy Metals by Some Fast Growing Halophytes and Maize Plants

Eid, M.A.

Soil Science Departments, Faculty of Agriculture, Ain Shams University, Hadayek Shoubra, Cairo, Egypt

ABSTRACT: Phytoremediation of soil heavy metal is emerging as a promising mechanism for decontaminating heavily metal-polluted soils. This study aimed to evaluate the phytoremediation potential of three fast-growing, high-biomass halophytic species and maize (Zea mays) for heavy metals. The highest shoot accumulation values for Cu were found in Spartina patens (17 mg kg\(^{-1}\) shoot DW) while, Zea mays accumulated 7 mg kg\(^{-1}\) shoot DW. The capacity of Ni accumulation in shoot of tested plants was ranked in descending order: Spartina patens, Sporobolus virginicus, and Zea mays. Despite of Z. mays had completely failed to translocate Cu into shoot but, it had the greatest phytoextraction potential, recording the lowest values of extractable metal in contaminated soil.

1. Introduction:
Agricultural soils in many parts of the world are slightly to moderately contaminated with heavy metals. This could be due to long-term use of excessive fertilizers, pesticides, fungicides, sewage sludge and bad water practices (Passariello et al., 2002 and Yadav, 2010). Zinc, copper and nickel are considered as soil pollutant metals due to their widespread occurrence and their acute effect on plants grown in such soils. In trace amount, they are essential elements for higher plants; however, in higher concentrations they are potentially toxic for plants. Phytotoxicity results in weak plant growth and yield depression. Daily consumption of heavy metals contaminated food poses a serious risk to human health (Dan et al., 2008 and Guala et al., 2010). Remediation strategies are therefore needed to clean up contaminated agricultural soils to produce safe foods for human consumption from these soils. Physicochemical methods have been widely used for remedying polluted soil especially at a small scale. However, they were not suitable for a large scale of remediation for its high cost and side effects. Recently, Phyto remediation was referred as biological phytoremediation (Chaney et al., 1997), involves the use of green plants to decontaminate soils, water and air. It is an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt et al., 1998). However, there are different categories of phytoremediation, including phytostabilization, phytoextraction, phytofiltration, phytovolatilization, phytodegradation depending on the mechanisms of remediation (Lone et al., 2008). Among which, phytoextraction and phytostabilization are the most reliable for heavy metals (Vamerali et al., 2010). Phytostabilization does not aim to remove contaminants from the soil, but decreasing their risks to human health and environment by reducing mobility and excluding metals from plants. In this case, a significant fraction of metals can be either stored at root level (Vamerali et al., 2009) or by strongly binding to many soil components (Turner and Dickinson, 1993).

Phytoremediation is usually defined as the utilization of plants to absorb, transport and concentrate metals from the soil into the harvestable shoots (Chen et al., 2004 and Manousaki et al., 2008). Preferably, plants should have among others, the following characteristics: (i) tolerant to high levels of metals; (ii) accumulating reasonably high levels of the metal in their above-ground tissues; (iii) rapid growth rates and (iv) producing reasonably high biomass (Alkorta et al., 2004). Nowadays, fast growing, high biomass production plant species that accumulate moderate levels of metal in their shoots are actively being tested for their metal phytoremediation potential. Interestingly, some of these fast growing, high biomass plant species are known to display a significant heavy metal tolerance.

Key words: Leptochloa fusca, Sporobolus virginicus, Spartina patens, Zea mays, Heavy metals, Phytoextraction, Phytoremediation.
Oat, ryegrass, etc. have been reported to tolerance and accumulate relatively high concentrations of metals in their tissues (Salt et al., 1995; Blaylock et al., 1997; Ebbs and Kochian 1997 & 1998 and Hernandez-Allica et al., 2008). Some authors considered that in some cases, a greater shoot biomass can more than compensates for lower shoot metal concentration. For example, Brassica juncea removed four-fold Zn more than the hyperaccumulator Thlaspi caerulescens from a contaminated soil. This was due primarily to that in 6 weeks, B. juncea produced 10 times biomass more than T. caerulescens (Ebbs and Kochian, 1997).

Despite more than 10 years of intensive research focused on the phytoextraction, very few commercial phytoextraction operations have been realized (Robinson et al., 2003). Therefore, selection of plant material is an important factor for successful field phytoremediation. This work aimed to evaluate the phytoextraction ability of three fast growing high biomass monocotyledon halophytes compared to Z. mays plant.

2. Materials and Methods

To evaluate and compare the potential of some halophyte grasses and maize plant for phytoremediation of heavy metals, a pot experiment was carried out in a greenhouse at Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The experiment was conducted as factorial ones including four plant species and three levels of heavy metals in five replicates arranged in a randomized complete block design. Four plant species including maize plants and three halophytes species, Leptochloa fusca (L.) Kunth, Sporobolus virginicus (L.) Kunth (smyrna) and Spartina patens (Aiton) Muhl. which obtained from the National Research Centre, Cairo, Egypt, were used in this study. Rhizome cuttings with 1-2 aerial stems were rooted in tap water for 7 days. All rooted cuttings (one cutting per pot) or ten seeds of maize were planted in a pot on July 1st 2009. Maize seedlings were thinned 5 days after emergence to five plants per pot. Three levels of heavy metal i.e. untreated (control), 25 mg Zn + 25 mg Cu + 25 mg Ni/kg soil) and 50 mg Zn + 50 mg Cu + 50 mg Ni/kg soil) were used. The combination of metals was based on Zn equivalent of 260 mg kg⁻¹ soil (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, the amounts of Zn, Cu and Ni used were 275 and 550 mg kg⁻¹ soil as Zn equivalent in the second and third level of heavy metal treatments, respectively. Soil sample was characterized by pH = 7.8, ECe = 2.5 dSm⁻¹, CaCO₃ = 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 8 kg sandy soil. Heavy metal salts (ZnSO₄ 7H₂O, CuCl₂ and NiCl₂ 6H₂O) were well mixed with the sandy soil in the pot. After planting, each pot was watered to keep moisture content approximately at 75% of water holding capacity. Seven days after sowing, each pot was irrigated with a modified nutrient solution (Arnon and Hoagland, 1940) till the end of the experiment.

The plant shoots were cut after 15 weeks except Leptochloa fusca was cut initially after 6 week then three times every 3 weeks. The dry weight of Leptochloa fusca is the sum of 4 successive cuts and the concentrations of metals in shoot are the average of 4 cuts. After the last harvest the soil samples were taken from each pot, air dried and then kept in plastic bags for analysis. Plant samples were dried at 70°C for 48h then 0.5 g of dry matter was wet ashed with the ternary acid mixture, HNO₃, HClO₄, H₂SO₄, the plant contents of Zn, Cu and Ni were determined using atomic absorption spectrophotometer (Varian Spectra AA20, Victoria, Australia).

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 and then were determined using atomic absorption spectrophotometer.

All parameters of soil and plant samples were analyzed statistically by multiple factor analysis of variance in randomized complete block design using Tukey’s multiple range test of significant at 5% level as described by Steel and Torrie (1980).

3. Results and Discussion

Plant biomass production

The analysis of variance clearly showed significant differences among the four tested plant species on the average of shoot dry matter production under different treatments (Table 1). Maize plants produced the highest significant shoot dry biomass followed by Leptochloa fusca then both of Sporobolus virginicus and Spartina patens. However, in the presence of metals, the shoot dry biomass for all tested plants significantly decreased compared to untreated plants (Table 1). Zn, Cu and Ni are essential elements, needed in a trace amount by higher plants and are involved in several metabolic processes, but in higher concentration they are potentially toxic for plants. The critical foliar metals concentration, in which the metal is shown to be toxic, varied between plant species. Most of plant species are sensitive to Zn in the range of 200 to 300 mg Zn kg⁻¹ shoot dry weight, but the critical
concentration of Cu ranged between 15 and 20 mg Cu kg\(^{-1}\) dry weight (Pahlsson, 1989). Meanwhile, the threshold values of shoot Ni concentration are usually in the range of 10-50 mg Ni kg\(^{-1}\) dry weight (Marschner, 1995). This may give us an explanation concerning the growth inhibition observed in our tested plants either under low or high contaminated rates. In particularly Ni was set at the critical toxic range in shoot tissues of all tested plants (Fig. 1). The long period of the experiment (15 weeks) may be was the second factor negatively affected on biomass production. In this respect, Eid and Eisa (2010) tested the effect of artificial pollution with 25 mg kg\(^{-1}\) soil of multiple Zn, Cu and Ni on Sporobolus virginicus and Spartina patens grown for 8 weeks. They reported that no growth inhibition on shoot dry biomass was occurred. However, the negative effect of heavy metals on shoot biomass production for maize and other grasses has been reported by several authors (Pahlsson, 1989; Mahmood et al., 2005 and Benimeli et al., 2010). The toxic effect of trace elements on plants is suggested due to their direct or indirect affects on the metabolic processes such as respiration, photosynthesis, CO\(_2\) fixation, gas exchange and other cellular processes (Vangronsveld and Clijster, 1994 and Mocquot et al., 1996). It is well known that Zn toxicity symptoms are the decrement of leaf chlorophyll content and rate of photosynthesis (Porter and Sheridan, 1981). At some stages in biosynthesis of chlorophyll, Zn is supposed to compete with Fe, or interferences with Fe-metabolism, where Zn is supposed to inhibit or reduce the capacity of roots which led to Fe-deficiency (Pahlsson, 1989). An excess of Cu can produce toxic effects on plants, such as inhibiting plant growth (Murphy et al., 1999). Also, Cu toxicity caused an inhibition of photosynthesis and respiration, the photosystem I is considerably more sensitive than photosystem II. Moreover, the activity of PEPCase enzyme, a key enzyme in C\(_4\) plants was significantly affected (Stiborova et al., 1986). The Ni element reduces maize growth by a reduction of root mitotic activity, and this probably because of direct action on the meristem or by reducing in carbohydrate transport from leaves to roots (Huillier et al., 1996).

Metals uptake and accumulation

Leptochloa fusca plants showed the highest significant values of shoot Zn accumulation (335 mg Zn kg\(^{-1}\) shoot DW) compared to all tested species. This value was three, four and eight times more than those found in Zea mays (103 mg Zn kg\(^{-1}\) shoot DW), Sporobolus virginicus (85 mg Zn kg\(^{-1}\) shoot DW) and Spartina patens (43 mg Zn kg\(^{-1}\) shoot DW), respectively, as shown in Fig. (2). On the other hand, the lowest residual extractable Zn was observed in contaminated soil cultivated with Zea mays plants which recorded values significantly equal to untreated plants. This was true either for low or high polluted levels. Meanwhile, both of Leptochloa fusca and Sporobolus virginicus successfully decreased soluble Zn in soil only at the lower contaminated rate (Fig. 4). With an exception of Spartina patens, it seems that the other three species had no problem with uptake, translocate and concentrate Zn from soil into their shoot, but their ability of metal translocation greatly varied among them. The highest phytoextraction rate was found by Leptochloa plants followed by Zea maise and Sporobolus plants, respectively, (Table 1 and Figs. 2&3). Concerning copper, only the three halophytic species succeeded to increase Cu translocation and accumulation in their aerial parts as the result of increasing Cu concentration rates in soil (Fig.5). Meanwhile, the Cu accumulation in maize shoot was stable by increasing Cu contamination rate compared with controls. The comparison between tested halophytes and maize plants for their efficiency in Cu foliar accumulation, it is clear that the Sporobolus virginicus, Leptochloa fusca and Spartina patens accumulated Cu in their shoots 4, 3 and 2 times higher than maize (Figs. 5&6). On the other hand, maize plant has the highest capacity to reduce the soluble Cu in polluted soil compared to tested halophytes (Fig. 7).

The highest Ni concentration was achieved by Leptochloa fusca (45 mg Ni kg\(^{-1}\) shoot DW) followed by Sporobolus virginicus (34 mg Ni kg\(^{-1}\) shoot DW) Spartina patens (20 mg Ni kg\(^{-1}\) shoot DW) and Zea mays (16 mg Ni kg\(^{-1}\) shoot DW) as shown in Table (1) and Fig. (8). Here again, maize plants successfully reduced soluble Ni concentration in contaminated soil over other species, arriving to the same level of control, but without effective translocation into aerial parts (Fig. 10).

Phytoremediation and more specifically phytoextraction, which involves using of plants to remove heavy metals from the soil into the harvestable above-ground biomass has been posed as a cost effective, environment friendly alternative restoration strategy for clean up of heavy metal contaminated soil (US EPA, 2001; Butcher, 2009 and Manousaki and Kalogerakis, 2009). Metal uptake and translocation from root to shoot is basically linked to the element speciation, plant species and other considerable factors. Results presented here indicated that maize plant had no problem with uptake, translocate and concentrating Zn from the contaminated soil but it failed to translocate and concentrate Cu in its shoot system (Figs.5&6).

In this concern, Mahmood et al. (2005) reported that the presence of Cu in culture medium
showed a stronger effect on root growth than shoot of maize plants while the reverse was true for Zn. Some preventive mechanisms were found in maize plants for reducing translocation of Cu from root to shoot (Benimeli et al., 2010). The distribution of two metals were completely different between root and shoot of maize plants, Cu seems to be concentrated in the roots while Zn appeared to be more diffusible than Cu (Mahmood et al., 2005). Also, there are numerous reports on several plants, their roots accumulated higher amounts of Cu than aerial parts (Lepp, 1981; Marschner 1995 and Mocquot et al., 1996). Therefore, low Cu concentration in the above-ground tissues of maize plants may be suggested either that is not taken up by plants but it strongly bind to many soil components to be hard to mobilize (Turner and Dickinson, 1993) or copper may not be transferred from roots into shoots (Marschner, 1995). Such a low Cu transfer to the aerial parts may be explained by a storage mechanism of Cu in root tissues (Khan, 2001) or by the low mobility of Cu in plants due to binding to the xylem (Nissen and Lepp, 1997). In this concern, Ait Ali et al. (2002) proposed that maize plant as a possible solution for stabilization of Cu in polluted soil. Phytostabilisation does not aim to remove contaminants from the soil, but reducing their risks to human health and environment. The establishment of green canopy in polluted soil has the effect of reducing the mobility of pollutants through water, wind erosion and water percolation. A significant fraction of metals can be stored at root level (Vamerali et al., 2009). Also, Ni seems to be accumulated in roots of maize, particularly at the root apex (Huillier, 1996).

The above presented results indicated that Leptochloa fusca had a greatest phytoextraction potentiality to accumulate Zn and Ni in its aerial organs. However, Cu translocation and accumulation in shoot only achieved by the three tested halophytes. The phytoextraction efficiency for Cu was generally ranked in descending order Sporobolus virginicus, Leptochloa fusca, Spartina patens.

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

<table>
<thead>
<tr>
<th>Analysis of variance</th>
<th>Dry weight</th>
<th>Plant concentrations</th>
<th>Plant uptake</th>
<th>Soil available</th>
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<tr>
<td></td>
<td>Zn</td>
<td>Cu</td>
<td>Ni</td>
<td>Zn</td>
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<td>** Type of plant **</td>
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<td>Heavy metals</td>
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<td>Interactions of plants X heavy metals</td>
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<td>** Main effect **</td>
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| Type of plant | Leptochloa | B | A | A | A | A | A | AB | AB | BC |
|               | Spartina   | C | C | A | B | C | B | C | A | A |
|               | Sporobolus | C | B | A | A | C | AB | BC | AB | A |
|               | Corn       | A | B | B | B | B | B | AB | AB | B |
|               | Control    | A | C | C | C | B | B | B | B | B |
| Level 1       | B           | B | B | B | B | AB | B | A | A | A |
| Level 2       | A           | A | A | A | A | A | A | A | A | A |

| Heavy metals | Control heavy metals | NS | DE | C | CD | BCD | AB | B | E | B | EF |
|             | Spartina heavy metals | NS | E | C | CD | D | B | B | DE | B | F |
|             | Sporobolus heavy metals | NS | E | C | CD | D | B | B | E | B | F |
|             | Corn heavy metals | NS | E | C | D | BCD | AB | B | E | B | F |
|             | Leptochloa X Level 1 heavy metals | NS | B | ABC | BC | B | AB | B | BCDE | AB | CDE |
|             | Spartina X Level 1 heavy metals | NS | DE | BC | BCD | D | AB | B | BCDE | AB | CDE |
|             | Sporobolus X Level 1 heavy metals | NS | CDE | ABC | BCD | CD | B | B | BCDE | A | CDE |
|             | Corn X Level 1 heavy metals | NS | DE | C | CD | BCD | AB | B | CDE | B | DEF |
|             | Leptochloa X Level 2 heavy metals | NS | A | AB | A | A | A | A | ABCD | A | BCD |
|             | Spartina X Level 2 heavy metals | NS | DE | ABC | BCD | D | AB | B | A | A |
|             | Sporobolus X Level 2 heavy metals | NS | CD | A | AB | BCD | AB | AB | A | AB |
|             | Corn X Level 2 heavy metals | NS | C | C | CD | BC | AB | B | BCDE | AB | ABC |

*, ** 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 Zn, Cu and Ni on the dry weight of different plants.

Fig. 2. Effect of Zn, Cu and Ni on the concentrations of Zn in different plants.

Fig. 3. Effect of Zn, Cu and Ni on the Zn uptake by different plants.
Fig. 4. Available soil Zn affected with different plants grown on the sandy soil treated with Zn, Cu and Ni.

Fig. 5. Effect of Zn, Cu and Ni on the concentrations of Cu in different plants.

Fig. 6. Effect of Zn, Cu and Ni on the Cu uptake by different plants.
Fig. 7. Available soil Cu affected with different plants grown on the sandy soil treated with Zn, Cu and Ni.

Fig. 8. Effect of Zn, Cu and Ni on the concentrations of Ni in different plants.

Fig. 9. Effect of Zn, Cu and Ni on the Ni uptake by different plants.
Fig. 10. Available soil Ni affected with different plants grown on the sandy soil treated with Zn, Cu and Ni.

Corresponding author
Eid, M.A.
Soil Science Department, Faculty of Agriculture, Ain Shams University, Hadayek Shobra, Cairo, Egypt
mohamedabceid@hotmail.com

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