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

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Abstract: Phytoextraction of heavy metal from contaminated soils is promising remediation technology. Till now, more than several hundreds of plant species have been used. However, phytoextraction using halophytes is still not extensively researched. This work was carried out to evaluate of phytoextraction potential for three fast growing halophytic grasses *Leptochloa fusca, Sporobolus virginicus* and *Spartina patens* vs *Zea mays* plants. The highest values of shoot accumulation were found in *Leptochloa fusca* to record 335 and 45 mg kg^{-1} DW for Zn and Ni compared with 103 and 16 mg kg^{-1} DW in maize plants. However, the value of Zn concentration in shoot of maize surpassed those found in *Sporobolus virginicus* (85 mg kg^{-1} DW) and in *Spartina patens* (43 mg kg^{-1} DW). Only the halophytic species succeeded to translocate Cu into their aerial parts. The highest Cu accumulation value was achieved by *Sporobolus virginicus* (25 mg kg^{-1} shoot DW) followed by *Leptochloa fusca* (21 mg kg^{-1} shoot DW) and *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 *Leptochloa fusca*, *Sporobolus virginicus, Spartina paten*s and *Zea mays*. Despite of *Z. mays* had completely failed to translocate Cu into shoot but, it had the greatest phytostablisation potential, recording the lowest values of extractable metal in contaminated soil.
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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 watering 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, Phytoremediation was referred as botanical bioremediation **(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)**.

Phytoextraction 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. In this concern, many grasses such as maize, barley, 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 compensate 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}, $CaCO_3$ = 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 8 kg sandy soil. Heavy metal salts ($ZnSO_4$ $7H_2O$, $CuCl_2$ and $NiCl_2$ $6H_2O$) 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, HNO_3, $HClO_4$, H_2SO_4, the plant contents of Zn, Cu and Ni were determined using atomic absorption spectrophotometer (Varian Spetra 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 Sheriden, 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 maize* 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.

Analysis of variance		Dry weight	Plant concentrations			Plant uptake			Soil available		
			Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni
Analysis of variance											
Type of plant		**	***	***	***	***	*	***	***	***	***
Heavy metals		***	***	***	***	***	*	***	**	**	***
Interactions of plants X heavy metals		NS	***	*	**	***	*	**	**	*	***
Main effect		Tukey's Multiple range test									
Type of plant	Leptochloa	B	A	A	A	A	A	A	AB	AB	BC
	Spartina	C	C	A	B	C	B	C	A	A	A
	Sporobolus	C	B	A	A	C	AB	BC	AB	A	AB
	Corn	A	B	B	B	B	AB	AB	B	B	C
Heavy metals	Control	A	C	C	C	B	B	B	B	B	B
	Level 1	B	B	B	B	B	AB	B	A	A	A
	Level 2	B	A	A	A	A	A	A	A	A	A
Interactions											
Leptochloa X Control heavy metals		NS	DE	C	CD	BCD	AB	B	E	B	EF
Spartina X Control heavy metals		NS	E	C	CD	D	B	B	DE	B	F
Sporobolus X Control heavy metals		NS	E	C	CD	D	B	B	E	B	F
Corn X Control 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	ABC	A	ABC
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	A
Sporobolus X Level 2 heavy metals		NS	CD	A	AB	BCD	AB	B	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.

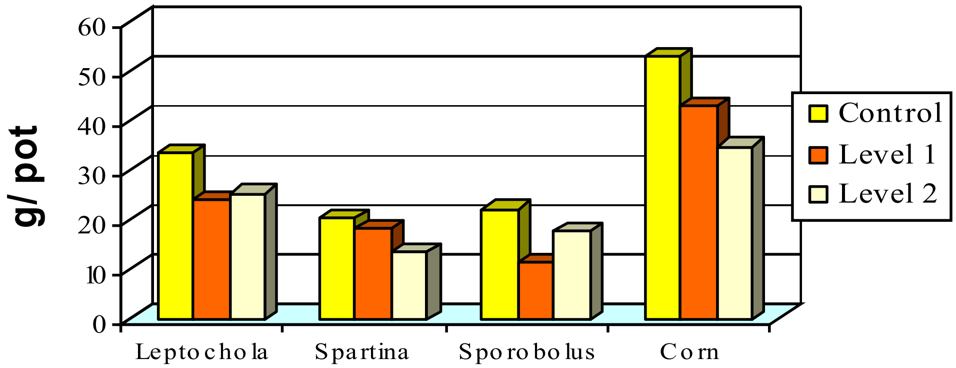

Fig.1. Effect of Zn, Cu and Ni on the dry weight of diffrent plants.

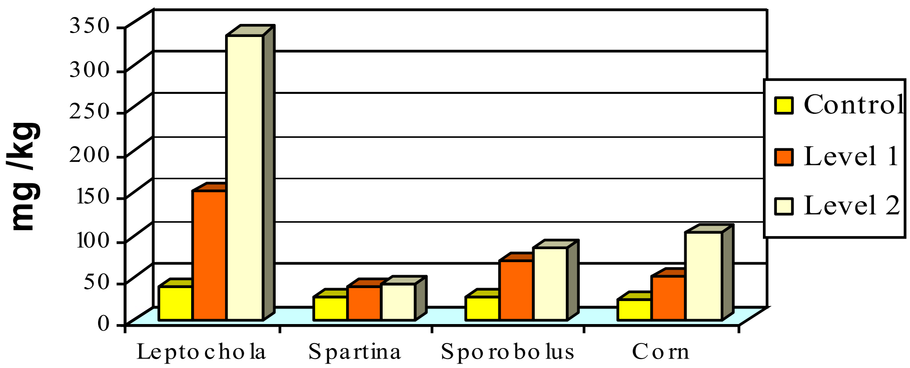

Fig.2. Effect of Zn, Cu and Ni on the concentrations of Zn in diffrent plants.

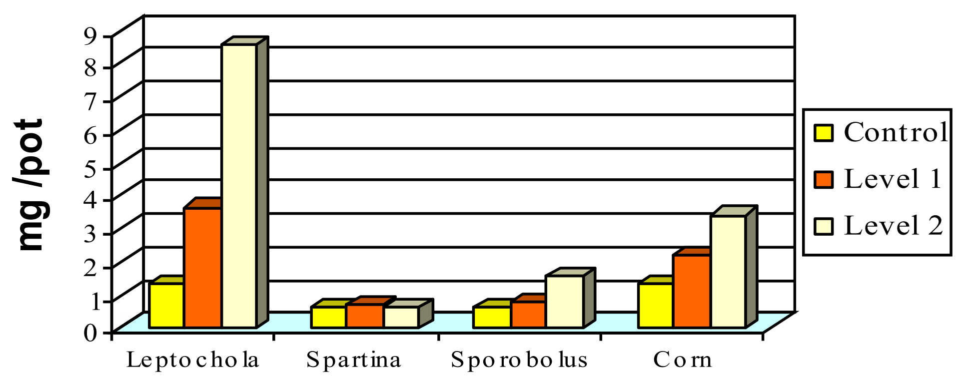

Fig.3. Effect of Zn, Cu and Ni on the Zn uptake by diffrent plants.

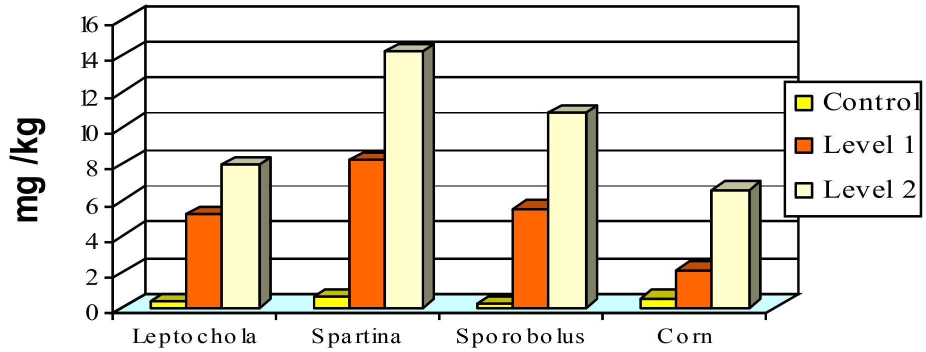

Fig.4. Available soil Zn affected with diffrent plants grown on the sandy soil treated with Zn, Cu and Ni.

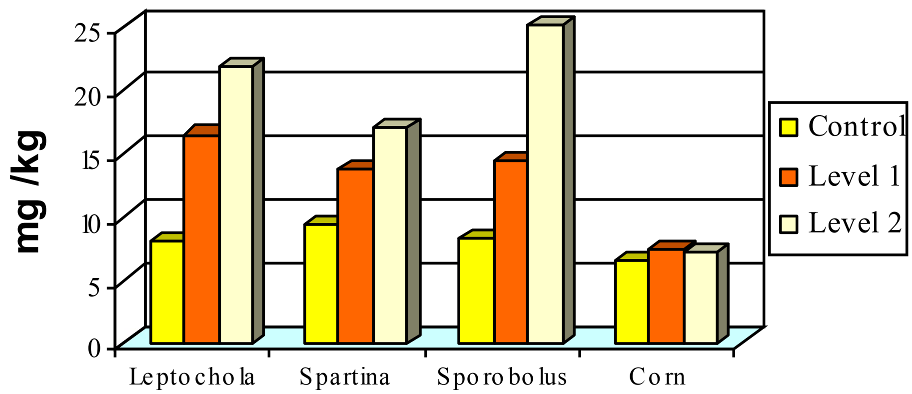

Fig.5. Effect of Zn, Cu and Ni on the concentrations of Cu in diffrent plants.

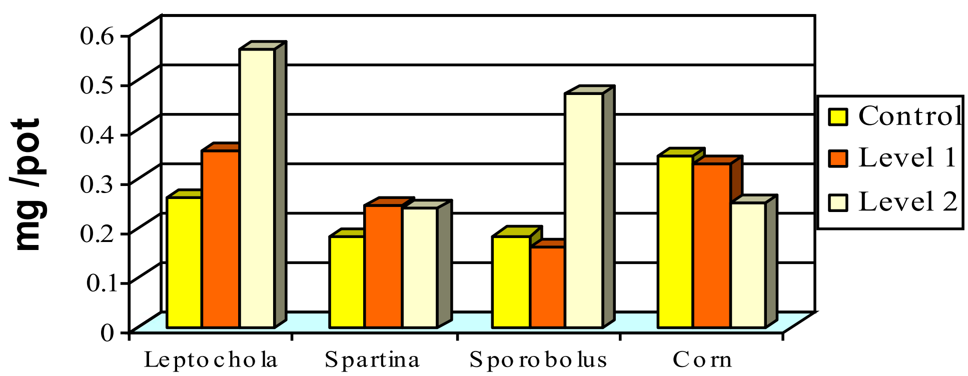

Fig.6. Effect of Zn, Cu and Ni on the Cu uptake by diffrent plants.

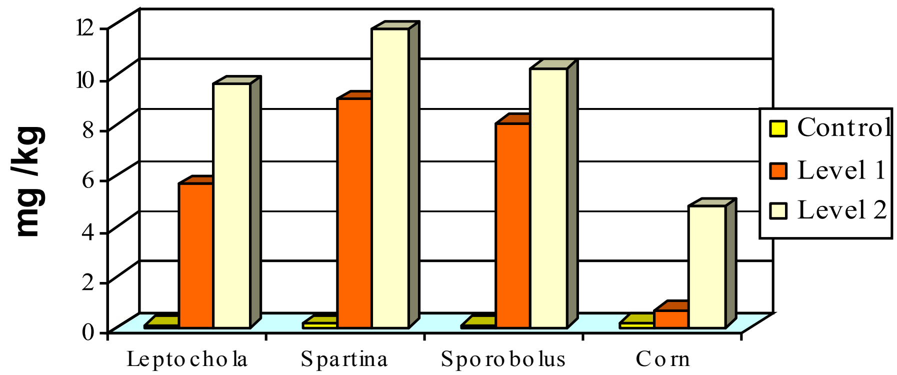

Fig.7. Available soil Cu affected with diffrent plants grown on the sandy soil treated with Zn, Cu and Ni.

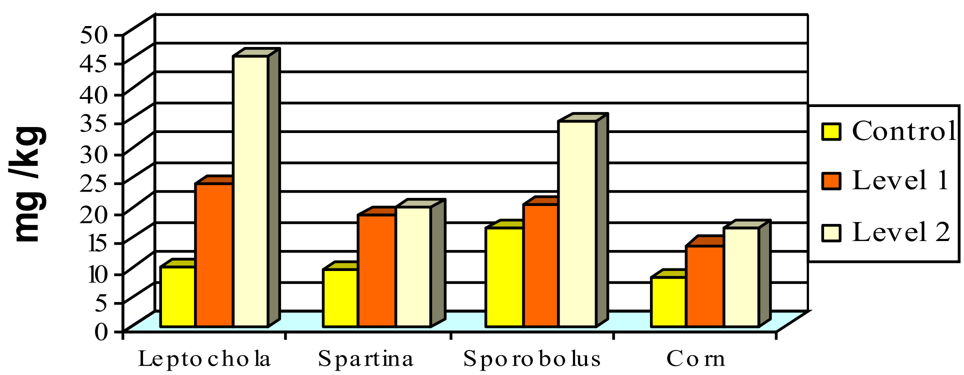

Fig.8. Effect of Zn, Cu and Ni on the concentrations of Ni in diffrent plants.

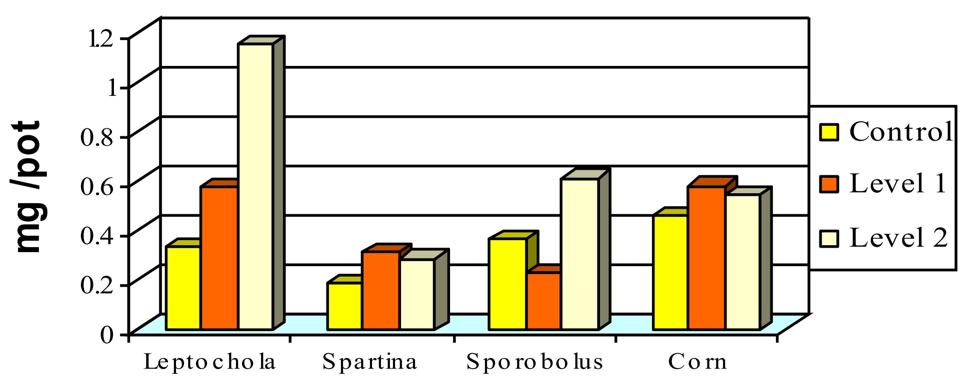

Fig.9. Effect of Zn, Cu and Ni on the Ni uptake by diffrent plants.

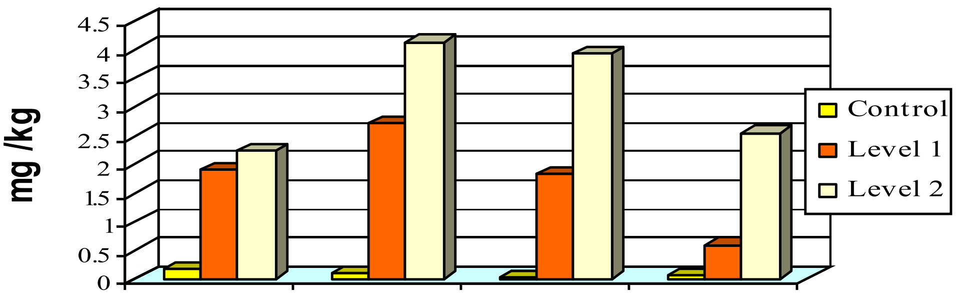

Fig.10. Available soil Ni affected with diffrent plants grown on the sandy soil treated with Zn, Cu and Ni.

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