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PHYTOREMEDIATION OF SOIL CONTAMINATED WITH ZINC AND LEAD BY USING *ZEA MAYS* L.

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Abstract

This study was carried out to investigate the potential of Corn (*Zea mays*) for phytoremediation of soil contaminated with lead (Pb) and zinc (Zn). The *Zea mays* L. cv. Giza 2, *Hordeum vulgare* cv. Giza 123 and *Lupinus termis* cv. Giza 1 species were planted in poted soil contaminated with lead and zinc. *Zea mays* was also cultivated with *Hordeum* and *Lupinus* treated with lead and zinc. The results showed that growth decreased by (16.7, 48.63 and 23.56%), photosynthetic pigments decreased by (10.18, 22.38 and 10.9%) and total-N decreased by (30.0, 27.27 and 13.64%) in *Zea, Hordeum* and *Lupinus*, respectively as compared to control. Proteins profile in shoots of tested plants revealed qualitative and quantitative changes. Co-cultivated *Zea* with *Hordeum* and *Lupinus* treated with lead and zinc improvement all parameters. The evidences provided by this experiment indicated that Corn acts as an effective accumulator to zinc and lead .

Introduction

High concentrations of heavy metals in soil may affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes. The most dangerous heavy metals are Pb, Hg, As, Cd, Sn, Cr, Zn and Cu (Wright 2007 and Gosh 2010). Zn2+ at high levels leads to chlorosis in young leaves, inhibits photosynthesis at various steps and decreases the growth and metabolic activity in various plant species (Vaillant *et al.*, 2005). Moreover, the reduction in nitrogen content under high zinc treatment had been shown by Sivasankar *et al.* (2012). Pb2+ inhibits some of metabolic activities in the plant (Hamid *et al.*, 2010). Eun *et al.* (2000) showed that a reduction in growth when exposed to increased levels of Pb resulted in the inhibition of cellular processes within root tips. In addition, responses of plants to Pb exposure include a decrease in root elongation and biomass (Fargasova 2001). Also photosynthesis is adversely affected by increasing levels of Pb through the distortion of chloroplasts, inhibition of electron transport, reduction of chlorophyll synthesis, disruption of Calvin's cycle enzymes and a reduction in carbon dioxide intake (Sharma and Dubey 2005). Saygideger *et al.* 2004, showed that there was a decrease in total-N content of *Typha latifolia* in parallel to increased Pb concentration. Plants that accumulate metals to high concentrations are sometimes referred to as ''hyperaccumulators'' (Visoottiviseth *et al.* 2002). Corn (*Zea mays*) is known to be a good accumulator of contaminants (Molgarzata and Andzej 2005). The maize plant has been even shown to accumulate certain heavy metals such as Pb (Pereira *et al.* 2007). The aim of this study is to assess the effect of lead and zinc on physiological and biochemical attributes of *Zea mays* L. cv. Giza 2, *Hordeum vulgaris* cv. Giza 123 and *Lupinus termis* cv. Giza 1, and the potential of *Zea mays* for phytoremediation of lead and zinc.

Material and Methods

Pure strains of *Zea mays* L. cv. Giza 2, *Lupinus termis* cv. Giza 1 and *Hordeum vulgare* cv. Giza 123 were obtained from Egyptian Agricultural Organization, Ministry of Agriculture, Egypt.

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The experiment was conducted at the Faculty of Education, Ain Shams University. Homozygous seeds were sterilized by 0.01 M $HgCl2$ solution for three minutes, washed thoroughly with distilled water and eight seeds were planted in each pot (3.5 kg/pot). The 1st group included two subgroups each contained *Zea* plants, first irrigated with tap water all through and the other *Zea* plants were irrigated after 10 days from sowing with aqueous solutions 100 mg/l Zn + 100 mg/l Pb. The 2nd group included three subgroups each contained *Lupinus* plants, the third subgroup contained *Zea* cultivated with *Lupinus* having 4 seeds each of *Zea* and *Lupinus*. The 3rd group included three subgroups, each contained *Hordeum* plants, the third subgroup contained *Zea* cultivated with *Hordeum* each pot containing 4 seeds of each of *Zea* and *Hordeum*. All pots were kept inside an open air wire house exposed to normal day length and natural illumination. Growth measurements were carried out at 32 days after sowing. Ten replicates were used for growth and triplicates for pigments, total-N and proline. Other samples were taken for the determination of certain mineral ions and protein.

The chlorophylls (Metzner *et al.* 1965) and total –N (Pirie 1955) as described by Hassanein (1977). Pb and Zn were determined in shoot and root using ICP inductively coupled Argon plasma, icap 6500 Duo using the method of AACC (1983) and that of proline by Bates *et al.* (1973). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total soluble protein (Laemmli (1970) as modified by Studier (1973). The obtained data were statistically following SAS- Programme (1982).

Results and Discussion

Pb and Zn stress caused a significant decrease in growth biomass of *Zea, Lupinus* and *Hordeum* as compared to control (Table 1). On the other hand, *Zea* plant cultivated with *Lupinus* and *Hordeum* plants decreased the inhibition of the growth characters in both plants under the Pb and Zn stress. It has been reported that Pb reduces root growth by restricting cell division (Eun *et al.* 2000) and cell elongation (Malkowski *et al.* 2002). Zeng-Bin Luo *et al.* 2010) also reported that high levels of Zn inhibited growth of *Jatropha* seedlings by interfering with normal cellular metabolic events and inducing visible injuries and physiological disorder. Moreover, the first visible damage due to excessive zinc was on root growth because of reduction in cell division (Prasad *et al.* 1999). The effects of Zn^2+ and Pb^2+ in combination leading to the injurious effects of the plants investigated which may suggest a synergistic effect between Zn^{2+} and Pb^{2+} on seedlings growth. Similar inhibitory effects in co-presence of metals have also been observed in other plant species (January *et al.* 2008, Israr *et al.* 2011). Guo *et al.* (2007) showed that the synergistic effects of heavy metal burdens are significantly more toxic than the individual heavy metal exposure.

Changes in chlorophyll a and b, carotenoids and total pigment contents in leaves of *Zea, Lupinus* and *Hordeum* plants illustrated significant decrease as compared to control in response to Pb and Zn treatments (Table 1). In addition, *Zea* plant cultivated with *Lupinus* and *Hordeum* increased the photosynthetic pigments in both plants under the Pb and Zn stress. Similar decrease in chlorophyll content under heavy metal stress was reported earlier in *Zea mays, Quercus palustrus* and *Acer rubrum* (Siedlecka and Krupa 1996).

Pb and Zn contents in roots and shoots of all plants in the test showed significant increases under stress with heavy metals (Table 2). Accumulation of Pb and Zn recorded in *Zea* plant was more than *Lupinus* and *Hordeum* plants. Total nitrogen content in shoots of *Zea*, *Lupinus* and *Hordeum* plants treated with Pb and Zn was significantly lower than their control ones. In contrast, the content of total nitrogen increased in treated plants when cultivated with *Zea mays,* which may refer to the decrease in proteolytic activity. This result is similar to Saadet *et al.* (2004), who reported a decrease in total N content of *T. latifolia* in parallel to increased Pb concentrations. Also, Godbold and Kettner (1991a) revealed that lead reduces the uptake and transportation of some nutrients in plants. Moreover, data (Table 2) showed a significant increase in proline content of shoots of all plants under Pb and Zn stress with those of the corresponding controls. On the other hand, cultivation of *Zea* plant with *Lupinus* and *Hordeum* plants caused a marked decrease of proline as compared to treated plants. Evidence suggested that the proline accumulation might contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles. Increase in proline content may be either due to de novo synthesis or decreased degradation or both (Kasai *et al.* 1998).

Table1. Effect of Zn and Pb on growth and photosynthetic pigment contents of *Zea, Lupinus* and *Hordeum* plants.

Samples	Shoot dry wt. (g)	Root dry wt. (g)	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Zea	1.626	0.374	25.13	7.53	6.73	39.69
Zea treated	1.370	0.296	22.85	6.47	6.33	35.65
L.S.D at 5%	0.118	0.0265	1.882	0.551	0.512	2.956
Lupinus	0.128	0.018	21.31	8.34	3.64	33.29
Lupinus treated	0.071	0.015	17.44	5.58	2.82	25.84
Lupinus treated cultivated with *Zea*	0.092	0.016	19.76	7.09	3.11	29.96
L.S.D at 5%	0.0081	0.0013	1.539	0.566	0.253	2.355
Hordeum	0.278	0.053	12.07	6.73	5.77	24.57
Hordeum treated	0.211	0.042	8.78	3.50	4.44	16.72
Hordeum treated cultivated with *Zea*	0.254	0.048	10.42	5.83	5.03	21.28
L.S.D at 5%	0.0147	0.0038	0.833	0.452	0.403	1.681

Table 2. Effect of Zn and Pb on Pb, Zn in shoot and root, total nitrogen and proline contents in shoot of *Zea, Lupinus* and *Hordeum* plants.

Samples	Pb (mg/g dry wt.)		Zn (mg/g dry wt.)		Total-N mgN/100g dry wt.	Proline content mg/100g fresh wt.
	Root	Shoot	Root	Shoot		
Zea	0.031	0.020	0.082	0.066	40	9.70
Zea treated	0.056	0.039	0.120	0.114	28	35.80
L.S.D at 5%	0.0036	0.0025	0.0081	0.0075	2.730	2.285
Lupinus	0.015	0.013	0.053	0.044	44	15.71
Lupinus treated	0.022	0.017	0.070	0.060	32	51.50
Lupinus treated cultivated with *Zea*	0.018	0.015	0.066	0.053	38	36.40
L.S.D at 5%	0.0015	0.0012	0.0050	0.0042	3.036	3.257
Hordeum	0.012	0.010	0.051	0.041	80	17.90
Hordeum treated	0.017	0.014	0.068	0.047	46	75.60
Hordeum treated cultivated with *Zea*	0.014	0.011	0.062	0.043	58	42.40
L.S.D at 5%	0.0012	0.00094	0.0048	0.0034	5.108	4.598

Variation in the SDS-PAGE banding pattern of proteins (Table 3) extracted from leaves of *Zea, Hordeum* and *Lupinus* plants in response to Pb and Zn stress showed the disappearance of certain bands and appearance of new bands or increase or decrease in the intensity of other protein bands. The extract of *Zea* plant was characterized by the presence of 13 common protein bands, molecular weights are; 127.65, 123.80, 119.64, 112.75, 102.75, 90.88,64.40, 59.71, 51.71, 43.20, 35.85, 28.09 and 8.9 kDa. There were two bands that disappeared in control and appeared with Pb and Zn stress at molecular weights 108.04 and 74.99 kDa. The total number of bands recorded with in the heavy metal treatments were found to be varied; i.e. the highest number (15 bands) was recorded in treated *Zea* plants, decreased to 13 bands scored in the control *Zea* plant. The extract of *Hordeum* plant was characterized by the presence of 11 common protein bands with molecular weights 127.65, 123.80, 119.64, 102.75, 90.88, 74.99, 59.71, 51.71, 43.20, 35.85 and 28.09 kDa. Metal stress caused the appearance of 2 protein bands of molecular weights 112.75 and 65.67 kDa, and the disappearance of one protein band with molecular weight 64.40 kDa. The combination of *Hordium* with *Zea* led to appearance of new 3 protein bands at molecular weights 115.1, 56.85 and 18.50 kDa as a defense mechanism for heavy metal stress.

Table 3. Effect of Zn and Pb on protein banding patterns of *Zea, Lupinus* and *Hordeum* plants.

No. of bands	M.Wt. kDa	Plant treated							
		1	2	3	4	5	6	7	8
1	127.65	+	+	+	+	+	+	+	+
2	123.80	+	+	+	+	+	+	+	+
3	119.64	+	+	+	+	+	+	+	+
4	115.10	-	-	-	-	-	-	-	+
5	112.75	+	+	+	+	+	-	+	+
6	108.04	-	+	+	+	+	-	-	-
7	102.75	+	+	+	+	+	+	+	+
8	95.10	-	-	-	-	-	-	-	-
9	90.88	+	+	+	+	+	+	+	+
10	74.99	-	+	+	+	+	+	+	+
11	65.67	-	-	-	+	-	-	+	+
12	64.40	+	+	+	+	+	+	-	-
13	59.71	+	+	+	-	-	+	+	+
14	56.85	-	-	-	+	+	-	-	+
15	51.71	+	+	-	-	+	+	+	+
16	50.28	-	-	+	+	-	-	-	-
17	43.2	+	+	+	+	+	+	+	+
18	35.85	+	+	+	-	-	+	+	+
19	32.75	-	-	-	+	+	-	-	-
20	28.09	+	+	-	-	-	+	+	+
21	18.50	-	-	+	+	+	-	-	+
22	8.90	+	+	+	+	+	+	+	-
Total number of bands		13	15	15	16	15	13	14	16

Lane1: *Zea* control
Lane 2: *Zea* treated
Lane 3: *Lupinus* control
Lane 4: *Lupinus* treated
Lane 5: *Lupinus* treated cultivated with *Zea*
Lane 6: *Hordeum* control
Lane 7: *Hordeum* treated
Lane 8: *Hordeum* treated cultivated with *Zea*

The highest number of bands (16 bands) was recorded in the *Hordium* treated plants and decreased to 13 bands in the control plant. In the case of *Lupinus* plant, there were 12 common protein bands with molecular weights 127.65, 123.80, 119.64, 112.75, 108.04, 102.75, 90.88, 74.99, 64.40, 43.20, 18.50 and 8.90 kDa. Metal stress induced the appearance of 2 bands with molecular weights 56.85 and 32.75 kDa, which acts as a defense protein to heavy metal stress, and disappearance of 2 bands whose molecular weights were 59.71 and 35.85 kDa. There was one band specific to metal stress only at molecular weight 65.67 kDa. There were also another band characterized to metal stress and a combination of *Lupinus* with *Zea* plants of molecular weight 51.71 kDa. The highest number of bands (16 bands) was recorded in the *Lupinus* treated plants and decreased to 15 bands in the control plant. The results of SDS-PAGE analysis for soluble proteins demonstrated that metals could affect protein pattern of the quantity of some bands that was changed in polluted plants, meaning that heavy metal stress could affect gene regulation and tend to decrease in some protein bands and increase in the other ones (Yousefi *et al.* 2011).

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