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PHYTOREMEDIATION OF LEAD FROM POLLUTED SOIL BY HYPERACCUMULATOR PLANTS

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ABSTRACT: *This experiment was conducted to study the phytoremediation by hyperaccumulator plants (Genotypes) [cowpea (Vigna unguiculata L.), sunflower (Helianthus annuus L. cv. Miac) and cockscomb (Celosia cristata L.)] in polluted soil by different concentrations of heavy metal such as lead (Pb) at levels 0, 50, 100, 200, 400, 800 and 1600 mg / 1000 g soil. During the plants accumulate the heavy metals from polluted soil these aspects were studied: Growth characters, some physiological and biochemical aspects, i.e photosynthetic pigments, water relations, total carbohydrates, total sugars, proline, activity of some enzymes, some mineral compositions, the accumulation of heavy metals in different plant parts, endogenous phytohormones as well as yield and its attributes of all crops. The accumulation of Pb metal inside the different crops at all levels of Pb led to decreased all vegetative growth characters, total and relative water content, transpiration rate, photosynthetic pigments, phenoloxidase activity, N, P, & K concentrations, endogenous phytohormons and yield and its components of all plants. Meanwhile it increased leaf water deficit, osmotic pressure, proline, peroxidase activity and concentration of Pb inside different plant organs. It could be recommended that, the use of hyperaccumulator plants led to decrease the concentrations of heavy metal Pb. The best crop we can use for this Purpose was cockscomb followed by sunflower. The extraction oil from sunflower seeds was cleared from any heavy metals accumulation.*

Key words: *lead; soil remediation; hyperaccumulator plants.*

INTRODUCTION

Heavy metal contamination is one of the most serious environmental problems limiting plant productivity and threatening human health. Amongst the substances that contribute anthropogenic ally to pollution of the biosphere, trace elements are one of the most toxic. Lead (Pb) is toxic metals of increasing environmental concern as they enter the food chain in increasingly significant amounts (Luptáková *et al.*, 2002 & Verma and Dubey, 2003). Restoration of soils contaminated with potentially toxic metals and metalloids is of major global concern (Shelmerdine *et al.,* 2009). As public awareness of Pb contamination increases, so have the questions concerning the safety of areas such as playgrounds, homes, and gardens. The greatest human concern regarding the toxicity or accumulation of heavy metals is directed towards small children. Their bodies and central nervous systems are developing rapidly and any exposure to Pb, even blood levels as low as 10 µg/L (0.1 ppm), can cause long-term health problems within many organ systems and mental and physical impairment (Succuro, 2010). It is interesting to examine toxic metal pollution of waters and soils is a major environmental problem, and most conventional remediation approaches do not provide acceptable solutions. Metal accumulation and toxicity of more than one metal in soil as in real conditions more metals are usually present in contaminated soil. Pb is known to influence each other's uptake by some plants when the two metals exist in the soil in significant amounts. This influence may be beneficial if it reduces uptake of metal by plants but may be detrimental if increased the uptake of the metal (Madyiwa *et al.*, 2004). The use of specially selected and engineered metal-accumulating plants for environmental clean-up is an emerging technology called phytoremediation. Three subsets of this technology are applicable to toxic metal remediation:

1- Phytoextration: the use of metal accumulating plants to remove toxic metals from soil.
2- Rhizofiltration: the use of plant roots to remove toxic metals from polluted water.
3- Phytostabilization: the use of plants to eliminate the bioavailability of toxic metals in soils. Biological mechanisms of toxic metal uptake, translocation and resistance as well as strategies for removing phytoremediation are also explained (Salt *et al.* 1995 and Chaney *et al.* 1997).
4- Plant roots can solubilize soil-bound toxic metals by acidifying their soil environmental with protons extruded from the roots (Crowley *et al.* 1991).

The aim of this work was study the uptake of lead from the soil supplemented with this metal separately, determine metal translocation between different plant parts. Moreover, to observe the effects of Pb on the growth, physiological, biochemical and yield of some crops during the experimental period, and select the best hyperaccumulator plant can be used for phytoremediation.

MATERIALS AND METHODS

The current investigation was conducted under controlled conditions of artificial pollution by heavy metal (Lead), at experimental farm of the Faculty of Agriculture, Menoufyia University during the two summer 2009 and 2010 seasons (the obtained results in the first season enough to view the results and the second season has quite similar) to study the phytoremediation by some hyperaccumulating plants [Sunflower (*Helianthus annuus* L. cv. Miac), Cowpea (*Vigna unguiculata* L. cv. Cream 7) and Cockscomb (*Celosia cristata* L.). (Baker *et al.*, 2000; Mcintyre, 2003 and Kopittke *et al*, 2007)] in polluted soil by different concentrations of heavy metal aforementioned.

Artificial pollution of the soil was done by adding lead salts in the form of $PbCl_2.2H_2O$ at concentrations of 0, 50, 100, 200, 400, 800 and 1600 mg (Pb) / kg soil (Ernst, 1996).

Plants taxonomy:

	Sunflower	Cowpea	Cockscomb
Order	Asterales	Fabales	Caryophyllales
Family	Compositae	Fabaceae (Papilionaceae)	Amaranthaceae

The seeds of plants aforementioned were obtained from the Crops Research Institute Agriculture Research Center in Cairo. Five seeds per pot were sown at 13th May in both seasons in pots 40 cm diameter, each pot filled with 15 kg of clay loamy soil. After 10 days the seedlings were thinned to one uniform seedling. The physical and chemical characteristics of experimental soil are shown in Table (1) according to Page (1982).

Weeds and best control as well as other agriculture practices were used whenever necessary.

Sampling: Plants were collected at 60 days from sowing, to determine the following data:

Studied characteristics:

1. **Growth characters:** Plant height (cm), Leaf area per plant (cm^2). (Fladung and Ritter, 1991), Relative growth rate (RGR), Net assimilation rate (NAR) (data collected in 40 and 60 and days from sowing to determine these parameters), dry weight of roots, shoots and totals dry weight / plant.
2. **Water relations:** Total water content (TWC) (Gosev, 1960 and Kreeb, 1990), Relative water content determination (RWC) (Barrs and Weatherley, 1962), Leaf water deficit (LWD), Osmotic pressure (Gosev, 1960) and Transpiration rate (Kreeb, 1990) were determined in fresh leaves.
3. **Photosynthetic pigments:** The pigment concentrations (chl.a, chl.b and carotenoids) were estimated using Wettestein's, 1957 formula cited in A.O.A.C., 1995 then calculated as mg / g D.wt.

Table (1): Some physical and chemical properties of experimental soil:

Properties	Value
Physical analysis	
Sand %	32.11
Silt %	35.43
Clay %	32.46
Texture	clay loamy
Chemical analysis	
PH	7.85
O.M. %	0.77
$CaCO_3$	1.49
Ec (mmhos/cm)	2.3
Soluble ions (meq/100 g soil)	
HCO_3^- Cl^- SO_4^{-2} Na^+ K^+ Ca^{+2} + Mg^{+2} Total N (100) Avail. P($Mg.g^{-1}$)	0.59 0.47 0.77 0.53 0.48 0.82 0.26 0.52
Heavy metals (mg/1000 g soil)	
Lead	1.02

4. Chemical analysis: Total carbohydrates and total sugars were measure in dry samples and determined spectro-calorimetrically using the phenol sulfuric acid method as described by Dubois *et al*. (1956) cited in A.O.A.C. (1995). All estimations were expressed as (mg/g D.wt.). Proline concentration was measured in fresh leaves using colorimeter according the method of Bates *et al*. (1973). Antioxidant enzymes activity: peroxidase and phenoloxidase activity in optical density/g (O.D./g fresh weight after 2 and 45 min), respectively were measured in the fresh leaves at 60 days from sowing using the methods described by Fehrman and Dimond (1967) and Broesh (1954),respectively. Nitrogen, phosphorus, potassium and sodium (%) in leaves, stems and roots were determined in the acid digest which was prepared by using a mixture of 5:1 sulphuric acid (H_2SO_4) : perchloric acid ($HClC_4$) respectively as a described by A.O.A.C. (1995). Extraction of heavy metals: Pb contents in plant roots, stems, leaves and seeds (at harvesting time only) at 60 and after harvesting time were determined by atomic absorption spectrophotometer (Model Perkin Elmer) and expressed as $\mu g/g^{-1}$ dry weight according to Cottenie *et al*., (1982). Form of Pb was extracted from the soil samples after harvesting time by sequential extraction (Walter and Cuevas, 1999)

5. **Determination of endogenous phytohormones:** Plant phytohormones in fresh shoots of sunflower, cowpea and cockscomb plants at 40 days from sowing and only grown under medium and high concentration of heavy metals were extracted and determined according to Wasfy *et al.* (1974)

6. **Yield and its components:** Sunflower: seed yield (g/plant), 1000 seeds weight, percentage of oil concentration in the seeds (oil percentage in the seeds was determined by Soxhlet extraction apparatus as described by A.O.A.C., 1995), total carbohydrates and protein contents in seeds were determined and heavy metal concentration in oil was determined. Cowpea: number of pods per plant, seed yield (g/plant), 1000 seeds weight, total carbohydrates and protein contents in seeds. Cockscomb: inflorescence length, inflorescence weight, seed yield (g/plant), 1000 seeds weight, total carbohydrates and protein contents in seeds.

All data collected were subjected to the standard statistical analysis following the proceeding described by Gomez and Gomez (1984) using the computer program of Costat Software (1985). The analyzed data then presented in tables.

RESULTS AND DISCUSSION

Growth characters:

Data recorded in Table (2), cleared that, the Pb concentrations from 50 to 1600 mg / kg soil caused a significant decrease in plant hight, leaf area, RGR, NAR and root, shoot dry weight of the treated plants. The highest reduction in these characters was recorded at the concentration 1600 mg / kg soil Pb at 60 days from sowing, as compared with the control. Similar observations have also been reported by Ruley *et al.* (2006) on *Sesbania* and Kovalchuk *et al.* (2005) on *Arabidopsis thaliana*, who found that, the analysis of the root length showed a dose dependent decrease in plants exposed to Pb (0 to 250 μM). The inhibitory effect of increased Pb concentrations on plants growth may be attributed to under severe lead toxicity stress, plants displayed obvious symptoms of growth inhibition, with fewer, smaller, and more brittle leaves having dark purplish abaxial surfaces (Islam *et al.*, 2007; Gupta *et al.*, 2009). Moreover, plant growth retardation as a result of lead exposure may be attributed to nutrient metabolic disturbances (Kopittke *et al.*, 2007) and disturbed photosynthesis (Islam *et al.*, 2008). Generally, heavy metals impede growth of plants, leading to smaller leaves that tend to be chlorotic (Kovacevic *et al.*, 1999; Sayed, 1999). These effects can be explained physiologically and biochemically as follow: The treatment with heavy metals can lead to the interruption of activities of several essential enzymes, various aspects of photosynthetic processes, uptake of essential nutrients, and the ultrastructure and water usage of cells (Sayed, 1999).

Water relations:

Data presented in Table (3) showed that, there was a remarkable gradual decrease in TWC, RWC and transpiration rate in leaves of sunflower, cowpea and cockscomb plants with increasing Pb levels from 50 to 1600 mg / kg soil. Meanwhile LWD and osmotic pressure were increased under the same contaminated levels of Pb at 60 days from sowing, respectively if compared with their control treatment (0 Pb). These results are in agreement with those obtained by El-Gamal and Hammad (2003) on tomato plants who reported that, lead (250 and 500 mg/l) negatively affected on water relations. In this respect, the plants are arranged according to the less affected as follows, cockscomb, sunflower then cowpea.

It can be concluded that, Pb have negative effects on some plant water relationships in the different plants under study. The pronounced negative effect was observed under the higher levels of lead treatment. It can be explained that as follows: According to Burzynski (1987) the inhibitory effect of heavy metals on some water relations may be attributed to their effects on transpiration and water content as a consequence of the harmful effect on transpiration system and stomatal structure. Heavy metals are thought to be one of the most dangerous stressors that occur in the environment (Shigeoka *et al,* 2002). There have been numerous studies on the toxicity of heavy metals, including lead and cadmium. Lead exerts adverse effects on water imbalance and alterations in membrane permeability (Singh *et al.,* 1997; Sharma and Dubey, 2005).

Table (2): Growth characters of the hyperaccumulating plants: sunflower, cowpea and cockscomb, grown in Pb-contaminated soil at 60 days from sowing during the growing summer season 2009.

Character / Pb (mg/kg soil)	Plant Height (cm)	Leaf area (cm^2)	RGR (mg/g/day)	NAR (mg/m2/day)	Dry weight (g)		
					root	stem	leaves
Sunflower							
0	139.0	202.13	1.85	0.33	0.81	15.27	16.08
50	137.5	201.01	1.83	0.31	0.72	14.76	15.48
100	132.5	196.12	1.79	0.29	0.71	13.99	14.70
200	131.1	193.74	1.71	0.29	0.63	12.30	12.93
400	128.5	188.63	1.65	0.28	0.60	11.45	12.05
800	111.3	182.33	1.53	0.26	0.47	10.14	10.61
1600	103.7	171.55	1.46	0.24	0.41	9.19	9.60
Mean	124.1	190.79	1.69	0.29	0.62	12.44	13.06
LSD 5%	3.001	0.902	0.024	0.003	0.071	0.875	0.099
Cowpea							
0	110.2	184.55	1.22	0.35	0.72	8.89	9.61
50	104.5	182.24	1.18	0.34	0.65	8.55	9.20
100	100.4	181.44	1.16	0.31	0.55	8.22	8.77
200	96.3	178.66	1.04	0.30	0.46	7.09	7.55
400	91.3	177.33	1.02	0.29	0.39	7.29	7.68
800	73.4	170.11	0.88	0.29	0.24	6.96	7.20
1600	62.6	161.12	0.68	0.27	0.19	5.92	6.11
Mean	91.10	176.49	1.03	0.31	0.46	7.56	8.02
LSD 5%	3.102	0.954	0.034	0.008	0.085	0.037	0.044
Cockscomb							
0	109.1	163.00	1.57	0.39	1.03	15.62	16.65
50	107.6	162.89	1.54	0.38	0.96	15.00	15.96
100	105.6	162.05	1.51	0.37	0.93	14.05	14.98
200	104.2	161.11	1.48	0.33	0.90	13.73	14.63
400	99.7	159.17	1.47	0.32	0.74	13.67	14.41
800	95.6	158.33	1.42	0.29	0.67	12.80	13.47
1600	91.4	155.33	1.33	0.27	0.59	12.05	12.64
Mean	100.7	160.27	1.47	0.34	0.83	13.85	14.68
LSD 5%	4.010	1.007	0.009	0.005	0.020	0.078	0.316

Table (3): Water relations of the hyperaccumulating plants: sunflower, cowpea and cockscomb, grown in Pb-contaminated soil at 60 days from sowing during the growing summer season 2009.

Character / Pb (mg/kg soil)	TWC (%)	LWD (%)	RWC (%)	Osmotic pressure (bar)	Transpiration rate (ml / cm^2.h)
Sunflower					
0	85.99	15.36	79.85	8.44	0.28
50	84.17	16.82	78.12	8.94	0.28
100	84.06	18.75	75.45	9.19	0.25
200	83.89	18.48	74.65	9.78	0.25
400	83.53	20.61	72.85	10.64	0.24
800	82.49	24.57	70.79	12.01	0.24
1600	81.12	27.92	68.55	12.73	0.23
Mean	83.61	20.36	74.32	10.25	0.25
LSD 5%	0.104	0.235	0.699	0.248	0.002
Cowpea					
0	84.12	14.11	82.69	8.06	0.29
50	83.98	14.34	81.51	8.27	0.29
100	83.82	15.20	81.41	8.86	0.28
200	82.99	16.61	77.49	9.06	0.26
400	81.56	18.54	75.13	11.29	0.25
800	79.88	21.56	74.01	11.46	0.25
1600	79.09	26.33	67.95	12.92	0.23
Mean	82.21	18.10	77.17	9.99	0.26
LSD 5%	0.139	0.207	1.094	0.202	0.007
Cockscomb					
0	86.94	14.65	81.85	7.52	0.29
50	86.75	16.07	80.90	7.90	0.28
100	86.57	18.10	79.84	8.62	0.28
200	86.09	18.90	78.30	9.13	0.27
400	85.73	20.14	77.01	9.72	0.25
800	85.26	21.16	76.40	10.17	0.24
1600	84.04	22.34	74.50	11.25	0.24
Mean	85.91	18.77	78.40	9.19	0.26
LSD 5%	0.175	0.797	0.601	0.341	0.001

Photosynthetic pigments:

From the obtained results in Table (4) showed that, Pb at 50, 100, 200, 400, 800 and 1600 mg / kg soil significantly decreased the concentrations of photosynthetic pigments (i. e. chlorophyll a, b, a + b and carotenoids) in leaves of sunflower, cowpea and cockscomb, meanwhile the ratios of a / b and a + b / carotenoids significantly increased with increasing of Pb levels. The decrease in chl. a, b, a + b and carotenoids at Pb level 1600 mg / kg soil was about -32.4, -41.3, -35.7 and 58.5% in sunflower, -38.5, -46.5, -41.4 and -67.1% in cowpea and -32.8, -35.2, -33.7 and -36.2% in cockscomb at 60 days from sowing respectively, if compared with their control plants. It is clearly that the decrease in photosynthetic pigments in these plants was nearly similar. These results are in agreement with those obtained by Selim (2000) on carrot plants who indicated that, a sharp decrease and degradation of chl.a and chl.b as well as total chlorophyll contents in plants grown in lead chloride and lead nitrate polluted soil (2000 and 4000 µg P/kg soil), Muhammad *et al*. (2008) on mung bean cultivars and Abdul Ghani (2010) on maize varieties came to the same results as well as Xiao Shu, *et al*. (2012) on *Jatropha curcas* who found that, Pb treatment had adverse effect on chlorophyll content, carotenoid content and Pb caused inhibition of leaf growth and photosynthesis.

Table (4): Photosynthetic pigments of the hyperaccumulating plants: sunflower, cowpea and cockscomb, grown in Pb-contaminated soil at 60 days from sowing during the growing summer season 2009.

Character / Pb (mg/kg soil)	60 Days					
	Chl. a (mg /g dwt)	Chl. b (mg /g dwt)	Chl. a+b (mg /g dwt)	Carote. (mg /g dwt)	Chl. a / b	Chl. a+b / carot.
Sunflower						
0	2.87	1.72	4.59	2.60	1.67	1.77
50	2.85	1.67	4.52	2.52	1.71	1.79
100	2.81	1.60	4.41	2.44	1.76	1.81
200	2.74	1.47	4.21	2.31	1.86	1.82
400	2.63	1.40	4.03	1.86	1.88	2.17
800	2.29	1.19	3.48	1.56	1.92	2.23
1600	1.94	1.01	2.95	1.08	1.92	2.73
Mean	2.59	1.44	4.03	2.05	1.82	2.05
LSD 5%	0.011	0.010	0.027	0.131	0.009	0.017
Cowpea						
0	3.51	1.98	5.49	2.70	1.77	2.03
50	3.31	1.81	5.12	2.51	1.83	2.04
100	3.27	1.79	5.06	2.48	1.83	2.04
200	3.12	1.57	4.69	1.83	1.99	2.56
400	2.79	1.40	4.19	1.61	1.99	2.60
800	2.61	1.29	3.90	1.08	2.02	3.61
1600	2.16	1.06	3.22	0.89	2.04	3.62
Mean	2.97	1.56	4.52	1.87	1.92	2.64
LSD 5%	0.005	0.003	0.002	0.034	0.007	0.074
Cockscomb						
0	2.59	1.48	4.07	2.32	1.75	1.75
50	2.56	1.45	4.01	2.28	1.77	1.76
100	2.53	1.43	3.96	2.24	1.77	1.77
200	2.42	1.35	3.77	2.13	1.79	1.77
400	2.09	1.16	3.25	1.82	1.80	1.79
800	2.05	1.14	3.19	1.75	1.80	1.82
1600	1.74	0.96	2.70	1.48	1.81	1.82
Mean	2.28	1.28	3.56	2.00	1.78	1.78
LSD 5%	0.032	0.030	0.069	0.008	0.005	0.021

As shown from our results, the concentration of photosynthetic pigments in the leaves of Pb-stressed plants under study significantly were decreased. The gradual decline in chlorophyll contents may be attributed to the gradual degradation of photosynthetic pigments (Muhammad *et al.*, 2008). Moreover, high Pb and Cu concentrations inside the leaf might have been high enough to directly inhibit chlorophyll synthesis (Sengar & Pandey, 1996). Lead accumulation in different organs is considered a general protoplasmic poison (Johnson & Eaton, 1980). Moreover, the key enzyme of chlorophyll biosynthesis δ-amino laevulinate dehydrogenase has been reported to be strongly inhibited by Pb (Prassad & Prassad, 1987).

Chemical composition:

The obtained results presented in Table (5) indicated that increasing Pb concentration in soil, decreased the total carbohydrates, total sugars and phenoloxidase activity in leaves of sunflower, cowpea and cockscomb plants at 60 days, meanwhile increased the proline concentration and peroxidase activity. The maximum reduction in total carbohydrates, total sugars and phenoloxidase activity was recorded at the level of Pb 1600 mg / kg soil. The observed results had similar trends like those obtained by Hamid *et al* (2010) on *Phaseolus vulgaris* plants found that, increasing lead acetate levels (25, 50 and 100 ppm) lead to several disruptions of *Phaseolus vulgaris* plants, which are reflected by reductions of carbohydrate content.

From the abetments results, it can be concluded that, the negative effect of Pb on carbohydrates levels of treated plants may be attributed to their deleterious effects on the photosynthetic process and water relation parameters inside plant tissues (Poskuta *et al.,* 1988 and Burzynski, 1987). proline concentrations increased with those of lead in the growth medium, and this increase is more relevant in roots than in coleoptiles (Saradhi, 1991). A detailed account has been studied about the production of the stress proteins (proline) whose synthesis and degradation is a very guarded phenomenon, regulated by a set of enzymes. Plants usually experience oxidative stress when they are exposed to Pb and other heavy metals (Dixit *et al*, 2001 – MacFarlane, 2003). A tissue Pb concentration greater than 30 ppm is toxic to most plant species (Xiong, 1997). The reactive oxygen species produced as a result of oxidative stress causes a variety of harmful effects in plant cells including lipid peroxidation. An increase in the H_2O_2 and MDA contents reflects that ROS caused lipid peroxidation in both ecotypes, which was more severe in the NME at 200μM (Islam *et al*, 2008). Increased level of H_2O_2 is produced either due to action of SOD (superoxide dismutase) on superoxide radicals or by direct formation in biochemical pathways viz., photorespiration. Lipid peroxides are formed by direct action of redox-active metals or indirectly by lipoxygenase-mediated lipid peroxidation by non-redox active metals (Mishra *et al*, 2006).

Mineral composition:

The concentrations of N, P and K in different plant organs of sunflower, cowpea and cockscomb plants (Table, 6) showed a decrease at the Pb levels from 50 to 1600 mg / kg soil. The decrease in N, P and K concentrations as a result of Pb contamination was more observed in roots than that in stems and leaves at all levels of Pb. The lowest N, P and K concentrations were recorded at the highest level of Pb (1600 mg / kg soil) if compared with their control plants. The obtained results are in agreement with those obtained by El-Ghinbihi (2000) on common bean, Wang *et al.*, (2011) on *Vallisneria natans* and Lamhamdi *et al.*, (2013) on wheat and spinach, who found that, Lead ($Pb(NO_3)_2$ at concentrations 1.5, 3, and 15 mM) is accumulated in a dose dependent manner in both plant species, which results in reduced growth and lower up take of all mineral ions tested, total amounts and concentrations of most mineral ions (Na, K, Ca, P, Mg, Fe, Cu and Zn) are reduced.

Table (5): Some chemical concentrations (total carbohydrates, total sugars, proline and enzymes activity) in leaves of the hyperaccumulating plants: sunflower, cowpea and cockscomb, grown in Pb-contaminated soil during the growing summer season2009.

Character / Pb (mg/kg soil)	60 Days		Proline conc. µg lucine /gm d.wt	Enzymes activity	
	Total carbohydrates (Mg/g d. wt)	Total sugars		Per-oxidase O.D./g Fwt. after 2 min.	Phenol-oxidase O.D./g Fwt after 45 min.
Sunflower					
0	144.55	17.67	245.46	0.73	0.95
50	140.47	16.47	289.17	0.80	0.87
100	140.15	15.64	464.50	0.87	0.82
200	132.07	12.85	518.90	0.96	0.79
400	128.85	12.31	682.43	0.99	0.75
800	97.97	11.91	861.92	1.01	0.68
1600	82.30	11.75	928.83	1.03	0.57
Mean	123.77	13.94	570.17	0.91	0.73
LSD 5%	3.887	0.154	37.756	0.018	0.019
Cowpea					
0	146.55	18.26	227.33	0.64	3.34
50	132.37	16.82	386.15	0.67	3.29
100	117.15	15.76	576.25	0.73	3.19
200	94.34	15.13	783.66	0.79	2.95
400	79.54	12.46	791.13	0.88	2.71
800	75.08	12.09	829.79	0.98	2.18
1600	70.22	11.73	935.18	1.06	1.94
Mean	102.18	14.61	647.07	0.82	2.80
LSD 5%	3.056	0.352	44.678	0.027	0.020
Cockscomb					
0	124.94	15.42	202.91	1.75	2.52
50	117.10	14.29	289.84	1.76	2.52
100	110.43	13.48	446.67	1.77	2.48
200	101.53	12.55	584.11	1.77	2.29
400	93.45	11.11	660.79	1.79	1.91
800	77.60	10.76	758.61	1.82	1.84
1600	68.39	10.53	835.88	1.82	1.64
Mean	99.06	12.59	539.83	1.78	2.17
LSD 5%	3.048	1.331	25.991	0.011	0.036

Table (6): Nitrogen, phosphorus and Potassium concentration (%) in root, stem and leaves of the hyperaccumulating plants: sunflower, cowpea and cockscomb, grown in Pb-contaminated soil at 60 days from sowing during the growing summer season 2009.

Character / Pb (mg/kg soil)	N (%)			P (%)			K (%)		
	root	stem	leaves	root	stem	leaves	root	stem	leaves
Sunflower									
0	2.04	3.38	3.66	0.24	0.34	0.49	2.40	3.03	3.30
50	1.85	3.02	3.22	0.22	0.33	0.43	2.35	2.99	3.20
100	1.44	2.82	2.94	0.22	0.29	0.40	2.30	2.88	3.09
200	1.28	2.54	2.76	0.17	0.26	0.39	2.21	2.82	3.03
400	1.17	2.41	2.68	0.15	0.25	0.38	2.16	2.80	3.00
800	1.10	2.05	2.21	0.13	0.25	0.33	2.09	2.75	2.91
1600	1.00	1.89	2.04	0.12	0.24	0.31	2.03	2.68	2.84
Mean	1.41	2.59	2.79	0.18	0.28	0.39	2.22	2.85	3.05
LSD 5%	0.065	0.129	0.077	0.009	0.008	0.010	0.045	0.019	0.025
Cowpea									
0	1.94	2.89	3.12	0.21	0.31	0.43	2.58	3.21	3.42
50	1.67	2.43	2.58	0.20	0.30	0.36	2.53	3.15	3.30
100	1.31	2.23	2.31	0.20	0.28	0.33	2.49	3.09	3.22
200	1.06	1.81	1.95	0.17	0.26	0.29	2.40	3.02	3.14
400	0.96	1.52	1.68	0.14	0.25	0.26	2.28	2.91	2.98
800	0.89	1.31	1.50	0.12	0.21	0.25	2.24	2.85	2.93
1600	0.73	1.27	1.42	0.10	0.20	0.22	2.16	2.79	2.83
Mean	1.21	1.92	2.08	0.16	0.26	0.31	2.38	3.00	3.12
LSD 5%	0.067	0.040	0.078	0.007	0.009	0.010	0.039	0.058	0.047
Cockscomb									
0	1.99	3.18	3.53	0.21	0.35	0.48	2.25	2.95	3.18
50	1.77	2.93	3.04	0.20	0.34	0.42	2.22	2.91	3.11
100	1.49	2.76	2.96	0.18	0.30	0.41	2.20	2.85	3.09
200	1.35	2.51	2.80	0.17	0.27	0.40	2.15	2.78	3.01
400	1.19	2.28	2.62	0.16	0.26	0.38	2.08	2.71	2.92
800	0.99	2.03	2.09	0.15	0.25	0.36	2.03	2.67	2.86
1600	0.87	1.84	2.06	0.14	0.24	0.34	1.99	2.63	2.82
Mean	1.41	2.51	2.73	0.17	0.29	0.40	2.13	2.79	3.00
LSD 5%	0.118	0.166	0.028	0.006	0.005	0.009	0.019	0.038	0.039

Results from multiple studies demonstrate that, nutrient content in plants is significantly affected by the presence of lead (Chatterjee *et al.* 2004 and Sharma and Dubey, 2005). Many studies indicated that lead exposure decreases the concentration of divalent cations (Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Fe^{2+}) in leaves of *O. sativa* (Chatterjee *et al.* 2004), *Medicago sativa* (Lopez *et al.* 2007) and *V. unguiculata* (Kopittke *et al.* 2007).

Accumulation of heavy metals in root, stem, leaf and seed:

From the obtained results in Tables (7 and 8) showed that, the accumulation of lead by root, stem, leaves and seeds of sunflower, cowpea and cockscomb plants increased with increasing the concentration of Pb from 50 to 1600mg / kg soil. On the other hand, the residual concentration of lead in soil after the harvest crop increased with increasing the level of Pb. The root in all crops under studying was recorded the main part in the accumulation. The highest accumulation in root, stem, leaves and seeds of crops under studying was recorded at the Pb level of 1600mg / kg soil. In this respect, the crops were arranged at the highest accumulated as follows, cockscomb > sunflower > cowpea These results are in agreement with those obtained by Sharma *et al.* (2006) on *Sesbania*, Islam *et al.* (2007) on *Elsholtzia argyi*, Liu *et al.* (2009) on wheat plants, Fazal *et al.* (2010) on maize plants and Yongsheng *et al.* (2011) showed that, the ability of Pb accumulation of tea plant, having a positive correlation with Pb (800, 1100, 1400, 1700 and 2100 mg/kg soil and in the sequence of root>stem>shoot. Root was the main part of tea plant to fix Pb.

Roots, which account for 20 – 50% of plant biomass, extract from the soil and deliver to the shoots most of the elements composing plant tissues, large proportion of Pb, Cd, Cu, Zn and Sr metals remains sorbet to solid soil constituents. To acquire these "soil-bound" metals, phytoextracting plants have to mobilize them into the soil solution. This so-called mobilization of "soil-bound" metal can be accomplished in a number of ways: 1- Metal-chelating molecules can be secreted into the rizosphere to chelate and solubilize "soil-bound" metal. 2- Roots can reduced "soil-bound" metals ions by specific plasma membrane bound metals reductases, which may increase metal availability Pea plants deficient in Fe or Cu have an increased ability to reduced Fe^{+} and Cu^{+2} wich is coupled with an increased uptake of Cu, Mn, Fe and Mg (Welch *et al,* 1993).

Endogenous phytohormones:

It can be noticed from data presented in Table (9) showed that, phytohormones concentrations in shoots of sunflower, cowpea and cockscomb plants was decreased at the medium level of Pb 100 mg / kg soil and the higher level of Pb 1600 mg / kg soil. The highest decrease in GA_3, IAA and kinetin concentrations was observed at 1600 mg / kg soil of Pb which reached about -57.4, -32.9 and -32.3% in sunflower, -67.1, -63.7 and -27.8% in cowpea and -45.40, -18.01 and -22.96% in cockscomb at 40 days from sowing, respectively. Meanwhile the same levels of Pb increased the content of ABA. The highest increase was recorded at the higher level of Pb by about 230.8% in sunflower, 213.3% in cowpea and 209.1% in cockscomb, respectively, if compared with their control plants. These results are in agreement with those findings reported by Chun-xia *et al.* (2010) on two species of *Ammopiptanthus* seedlings who indicated that, with the increase in Pb^{2+} concentration (20-1500 $mg \cdot L^{-1}$), the IAA decreased significantly; and only under the high density (more than 1000 mg·L-1) of Pb^{2+}, the GA_3 was affected by it; the ABA did not change regularly.

Abiotic stresses can also impede auxin transport by altering the pH in the plant apoplast (Murphy, 1998), or by altering the concentrations of phenolics such as quercetin and kaempferol, which can act as endogenous auxin transport inhibitors (Taylor and Grotewold, 2005). Alternatively, stress can impact on auxin stability. Peroxidases catalyse the oxidative degradation of IAA with the help of H_2O_2 (Gazaryan, 1998). Auxin plays a key role in shaping plant architecture, and it mediates responses to a broad range of external signals (Van Den Bussche and Van Der Straeten, 2004). However, the literature contains numerous examples of other hormones involved in the response to specific stress conditions.

Table (7): Accumulation of heavy metal (µg/g^{-1} dry wt.) by root, stem and leaves of the hyperaccumulating plants: sunflower, cowpea and cockscomb grown in Cd-contaminated soil at 60 days from sowing and after harvesting during the growing summer seasons 2009.

Character / Cd (mg/kg soil)	60 Days			After harvesting			
	root	stem	leaves	Root	Stem	Leaves	Seeds
	Sunflower						
0	0.07	0.01	0.03	0.07	0.02	0.05	0.01
10	0.92	0.09	0.33	1.92	0.17	0.68	0.61
20	1.87	0.23	0.32	2.70	0.37	1.21	0.77
40	3.46	0.66	1.26	4.83	1.00	2.14	1.24
80	9.07	1.73	3.31	14.82	2.83	5.41	1.51
160	22.36	4.26	8.16	33.32	6.35	12.17	2.18
320	65.13	17.06	22.92	68.94	21.99	28.57	3.82
Mean	14.70	3.43	5.19	18.09	4.68	7.18	1.45
	Cowpea						
0	0.05	0.01	0.02	0.10	0.03	0.05	0.00
10	0.27	0.05	0.10	0.54	0.09	0.19	0.51
20	0.51	0.14	0.32	1.20	0.24	0.55	0.64
40	1.85	0.31	0.87	3.05	0.40	1.28	1.05
80	7.80	1.08	2.46	10.56	1.91	4.11	1.27
160	15.62	3.45	4.09	16.47	5.65	10.02	1.87
320	25.23	9.01	18.05	45.89	12.77	24.45	3.29
Mean	7.33	2.01	3.70	11.12	3.01	5.81	1.23
	Cockscomb						
0	0.08	0.03	0.04	0.08	0.03	0.05	0.01
10	1.08	0.17	0.23	2.26	0.35	0.46	0.62
20	1.92	0.44	1.16	3.58	0.71	2.16	0.82
40	3.76	1.04	2.04	7.75	2.22	4.64	1.35
80	17.62	4.00	6.26	14.25	7.18	10.68	1.62
160	30.49	10.09	11.39	36.94	11.27	19.56	2.50
320	67.98	16.98	33.16	74.76	23.74	40.78	4.41
Mean	17.56	4.68	7.75	19.95	6.50	11.19	1.62
LSD 5%	1.802	0.548	3.921	1.462	0.337	1.448	0.010

Table (8): Accumulation of heavy metal (µg/g^{-1} dry wt. and %) by the hyperaccumulating plants: sunflower, cowpea and cockscomb grown in Pb-contaminated soil and the residual of heavy metals in soil after harvesting during the summer season 2009.

Character / Pb (mg/kg soil)	Season 2009			
	Total amount per plant (µg)	Total amount per plant (%)	Residual conc. in soil per pot (µg)	Residual conc. in soil per pot (%)
Sunflower				
0	14.72	0.00	0.21	0.00
50	410.87	54.78	320.13	42.68
100	1140.33	76.02	299.67	19.98
200	1755.49	58.52	1178.24	39.27
400	3611.20	60.19	2298.41	38.31
800	5862.48	48.85	6074.61	50.62
1600	9909.77	41.29	13995.48	58.31
Mean	3243.55	48.52	3452.39	35.60
Cowpea				
0	11.50	0.00	0.33	0.00
50	563.41	75.12	179.97	24.00
100	925.54	61.70	562.27	37.48
200	1559.15	51.97	1399.49	46.65
400	2216.86	36.95	3709.56	61.83
800	2882.04	24.02	9101.33	75.84
1600	5158.61	21.49	18822.32	78.43
Mean	1902.44	38.75	4825.04	46.32
Cockscomb				
0	13.94	0.00	0.14	0.00
50	716.86	95.58	11.65	1.55
100	1407.78	93.85	64.15	4.28
200	2851.93	95.06	129.42	4.31
400	5761.29	96.02	219.87	3.66
800	11260.28	93.84	697.48	5.81
1600	22922.80	95.51	1003.26	4.18
Mean	6419.27	81.41	303.71	3.40

Table (9): Endogenous phytohormones content (µg / 100 g F.W.) in shoots of the hyperaccumulating plants: sunflower, cowpea and cockscomb grown in Pb-contaminated soil at 40 days from sowing during the growing summer season 2009.

Character / Pb (mg/kg soil)	GA_3	IAA	Kinetin	ABA
Sunflower				
0	18435	1155	2653	13
100	13691	1002	2271	27
1600	7864	775	1795	43
Cowpea				
0	17233	1070	3108	15
100	11452	713	2572	34
1600	5679	388	2244	47
Cockscomb				
0	18818	1216	3157	11
100	15586	1159	2951	19
1600	10277	997	2432	34

Yield and its components:

Sunflower:

Data presented in Table (10) showed that seed yield, 1000 seeds weight, percentage of oil in the seeds, total carbohydrates and protein contents in seeds were decreased at all treatments of Pb, and the extraction seed oil was clean from Pb contamination. Similar findings have been demonstrated by Singh and Agrawal (2007) on *Beta vulgaris* who found that, increase in heavy metal (Ni, Cd, Cu, Cr, Pb and Zn) concentration in foliage of plants grown in sewage sludge-amended soil caused unfavorable changes in physiological and biochemical characteristics of plants leading to reductions in yield and Adewole *et al.* (2010) on sunflower found that, better yield of plants was obtained under non-polluted soil condition compared with grown under Pb-contaminated soil (0, 250, 500, 750, 1000 mg kg^{-1}).

Cowpea:

Concerning the effect of the Pb treatments on yield components, data presented in Table (11) showed that, all Pb levels 50, 100, 200, 400, 800 and 1600 mg / kg soil caused a decrease in number of pods per plant, seed yield, 1000 seeds weight, total carbohydrates and protein contents in seeds when compared with their control. The obtained results are similar to those recorded by Georgieva *et al.* (1997) on radish, pea and pepper plants found that, at higher levels of Pb (3000 ppm), growth and yield of radish, pea and pepper plants are reduced parallel to the enhancement of Pb concentrations, and El-Ghinbihi (2000), who showed that, the yield of common bean varieties, as represented by number and weight of pods per plant as well as number and weight of seeds per pod was significantly decreased by heavy metal treatments (lead at 0.1, 0.5 and 1mM).

Cockscomb:

Data presented in Table (12), cleared that, all levels of Pb caused a significant decrease in inflorescence length, inflorescence dry weight, seed yield, straw yield, 1000 seeds weight, total carbohydrates and protein contents in seeds. The reduction in these characters at the levels of 1600 mg / kg soil was the highest reduction compared the other levels of Pb as compared with the control. The obtained results are accordance with those reported by Selim (2000) on carrot plants found that, the root and top as well as total yield were negatively affected by Pb treatments and were severely in the polluted soil with lead chloride and lead nitrate at rates 2000 and 4000 µg P/kg soil.

Table (10): Yield and its components of the hyperaccumulating plant: sunflower grown in Pb-contaminated soil after harvesting during the growing summer season 2009.

Character / Pb (mg/kg soil)	Season 2009					
	Seed yield (g/plant)	1000 seeds wt. (g)	Oil in seeds (%)	Heavy metal in oil ($\mu g/g^{-1}$ dry wt.)	Total carbohydr. in seed (%)	total protein in seed (%)
0	5.82	48.18	42.46	0.00	28.91	26.33
50	5.15	37.45	41.92	0.00	28.56	25.45
100	4.21	35.65	41.52	0.00	27.84	25.15
200	2.71	33.28	39.54	0.00	27.17	24.80
400	2.40	29.78	39.24	0.00	27.03	24.44
800	1.56	17.98	39.07	0.01	26.48	24.26
1600	1.37	14.21	38.25	0.02	26.25	24.10
Mean	3.32	30.93	40.37	0.004	27.46	24.93
LSD 5%	0.167	2.574	0.792	ns	0.193	0.148

Table (11): Yield and its components of the hyperaccumulating plant: cowpea grown in Pb-contaminated soil after harvesting during the growing summer seasons 2009.

Character / Pb (mg/kg soil)	Season 2009				
	Pods number/ plant	Seed yield (g/plant)	1000 seeds wt. (g)	Total carbohydr. in seed (%)	total protein in seed (%)
0	12.66	25.08	218.00	54.95	29.86
50	10.00	23.51	207.65	54.27	29.81
100	9.66	20.62	194.74	53.99	29.73
200	9.33	17.57	190.12	53.91	29.65
400	8.33	9.84	183.85	53.75	29.49
800	7.66	6.55	172.91	53.28	29.25
1600	7.00	5.94	166.58	53.10	28.96
Mean	9.23	15.59	190.55	53.89	29.54
LSD 5%	0.289	0.556	3.757	0.079	0.048

Table (12): Yield and its components of the hyperaccumulating plant: cowpea grown in Pb-contaminated soil after harvesting during the growing summer seasons 2009.

Character / Pb (mg/kg soil)				Season 2009		
	Inflorescenc-e length (cm)	Infloresce-nce weight (g)	Seed yield (g/plant)	1000 seeds wt. (g)	Total carbohydr. in seed (%)	Total protein in seed (%)
0	14.45	1.93	0.81	0.735	59.11	32.53
50	14.33	1.77	0.71	0.693	59.09	32.47
100	13.57	1.68	0.66	0.669	59.07	32.45
200	11.56	1.49	0.56	0.634	59.03	32.37
400	10.82	1.32	0.47	0.556	59.00	32.35
800	9.75	0.98	0.41	0.482	58.96	32.33
1600	7.94	0.96	0.38	0.415	58.87	32.29
Mean	11.77	1.45	0.57	0.598	59.02	32.40
LSD 5%	0.118	0.026	0.027	0.038	0.017	0.015

It could be concluded that, yield and its components of sunflower, cowpea and cockscomb plants were reduced as a result of application Pb. This reduction may be attributed to as shown from our results, the Pb not only have been inhibited plant growth, water uptake, disruption in chemical compositions, but also increased their concentration in roots, stem and leaves leading to negative effect on yield and its components. In addition, IAA, GA_3 and Kinetin were decreased, whereas ABA was increased under Pb-contaminated conditions in sunflower, cowpea and cockscomb plants as shown from our results, thus the plant growth as well as the yield decreased. In this respect, Singh and Agrawal, (2007) indicated that increasing heavy metal (Ni, Cd, Cu, Cr, Pb and Zn) concentration in foliage of plants grown in sewage sludge-amended soil caused unfavorable changes in physiological and biochemical characteristics of plants leading to reduction in yield .

REFERENCES

Abdul Ghani (2010). Effect of Lead Toxicity on Growth, Chlorophyll and Lead (Pb^{+2}) Contents of Two Varieties of Maize (*Zea mays* L.). Pakistan Journal of Nutrition, 9 (9): 887-891.

Adewole, M. B., O. O. Awotoye, M. O. Ohiembor and A. O. Salami (2010). Influence of mycorrhizal fungi on phytoremediating potential and yield of sunflower in Cd and Pb polluted soil. J. of Agric. Sci., 55 (1): 17-28.

A.O.A.C. (1995). Association of Official Agriculture Chemists. Official Methods of Analysis. 16th Ed. A.O.A.C. Virginia, D.C., USA.

Baker, A. J. M., S. P. McGrath, R. D. Reeves and J. A. C. Smith (2000). Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils, In: Phytoremediation of Contaminated Soil and Water, Terry N, Bañuelos G, (Eds.), pp. 85-107, CRC Press LLC, USA.

Barrs, H. D. and P. E. Weatherley (1962). Arc examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci., 15: 413 - 428.

Bates, L. S., R. P. Waldem and 1. D. Teare (1973). Rapid determination of free proline under water stress studies Plant and Soil, 39: 205 - 207.

Broesh, S. (1954). Colorimetric assay of phenoloxidase. Bull. Soc. Chem., Biol., 36: 711-713.

Burzynski, M. (1987). the uptake and transpiration of water and the accumulation of water and the accumulation of lead by plants growing on lead chloride solution. Acta. Soc. Bot., 56: 271-280.

Chaney, R. L., M. Malik, Y. M. Li, S. L. Brown, E. P. Brewer, J. S. Anjel and A. J. Baker (1997). Phytoremediation of soil metals. Current Opinion in Biotechnology, 8(3): 279-284.

Chatterjee, C., B. K. Dube, P. Sinha and P. Srivastava (2004). Detrimental effects of lead phytotoxicity on growth, yield, and metabolism of rice. Commun Soil Sci Plant Anal., 35(1-2): 255-265.

Chun-xia, Z., C. Bo-lang, J. Hong-tao, S. Jian-dong and P. Xin-yan (2010). Comparative analysis of endogenous hormones between two species of *Ammopiptanthus* seedlings around germination and under Pb^{2+}Stress. Spectroscopy and Spectral Analysis, 30 (3): 820-824.

Costat, Software (1985). Costat 6311Windowes by Roland Sneif, Internet.

Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlyck (1982). Analytical Problems and methods in chemical plant and soil analysis. Hand book Ed.A.Cottenie, Gent, Belgium.

Crowley, K. D., M. Cameron and R. L. Schaefer (1991). Experimental studies of annealing etched fission tracks in fluorapatite. Geochimica et Cosmochimica Acta, 55: 1449-1465.

Dixit, V., V. Pandey and R. Shyam (2001). Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum*), J. Exp. Bot. 52: 1101-1109.

Dubois, M., A. Gilles, K. J. Hamilton, P. R. Rebers and P.A. Smith (1956). Colorimetric method for determination of sugar and related substances. Anals. Chem., 28: 350.

El-Gamal, S. M. and S. A. A. Hammad (2003). counteracting the deleterious effects of lead and cadmium on tomato plants by using yeast, garlic and eucalyptus extracts. Minufiya J. Agric. Res. 28(3): 737-755.

El-Ghinbihi, F. H. (2000). Growth, chemical composition and yield in some common bean varieties as affected by cadmium and lead levels. Minufiya J. Agric. Res. 25(3): 603-625.

Ernst, W. H. O. (1996). Bioavailability of heavy metals and decontamination of soil by plants. Appl. Geochem., 11:163-167.

Fazal, H., B. Asghari and P. F. Michael (2010). The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. Chemosphere, 80: 457-462.

Fehrman, H. and A. E. Dimond (1967). Peroxidase activity and phytophthora resistance in different organs of the potato. Plant pathology, 57: 69-72.

Fladung, M. and E. Ritter (1991). Plant Leaf Area Measurements by Personal Computers. Journal of Agronomy and Crop Science, 111(1): 19-07.

Gazaryan, I. G. (1998). Identification of the skatolyl hydroperoxide and its role in the peroxidase-catalyzed oxidation of indol-3-yl acetic acid. Biochem. J., 333: 223-232.

Georgieva, V., C. Tasev and G. Sengalevitch (1997). Growth, yield, lead, zinc and cadmium content of radish, pea and pepper plants as influenced by level of single and multiple contamination of soil. III. Cadmium. Bulg. J. Plant Physiol., 23(1–2): 12-23.

Gomez, K. A. and A. A. Gomez (1984). Statistical procedures for agricultural research. 2^{nd} ed. Jahn Wiley Sons, New York, U.S.A. pp. 680.

Gosev, N. A. (1960). Some methods in studying plant water relation. Leningrad Acad. of Sci. U.S.S.R.

Gupta, D., F. Nicoloso, M. Schetinger, L. Rossato, L. Pereira, G. Castro, S. Srivastava and R. Tripathi (2009). Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. J Hazard Mater, 172(1): 479-484.

Hamid, N., N. Bukhari and F. Jawaid (2010). Physiological responses of *Phaseolus* vulgaris to lead concentrations. *Pak. J. Bot.,* 42(1): 239-246.

Islam, E., D. Liu, T. Li, X. Yang, X. Jin, Q. Mahmood, S. Tian and J. Li (2008). Effect of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*. J. of Hazardous Materials, 154: 914–926.

Islam, E., X. Yang, T. Li, D. Liu, X. Jin and F. Meng (2007). Effect of Pb toxicity on root morphology, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*. J. of Hazardous Materials, 147: 806–816.

Johnson, M. S. and J. W. Eaton (1980). Environmental contamination through residual trace metal dispersal from a derelict lead-zinc mine. J. Environ. Qual., 9: 175–179.

Kopittke, P. M., C. J. Asher, R. A. Kopittke and N. W. Menzies (2007). Toxic effects of Pb2+ on growth of cowpea (*Vigna unguiculata*). Environ Pollut., 150(2): 280-287.

Kovacevic, G., R. Kastori and L. J. Merkulov (1999). Dry matter and leaf structure in young wheat plants as affected by cadmium, lead and nickel. Biologia Plantarum, 42(1):119-123.

Kovalchuk, I., V. Titov, B. Hohn and O. Kovalchuk (2005). Transcriptome profiling reveals similarities and differences in plant responses to cadmium and lead. Mutation Res., 570: 149–161.

Kreeb, K. H. (1990). Methoden Zur Pflanzenokologie und Bioindikation Gustav Fisher, Jena, 327 pp.

Lamhamdi, M., O. El Galiou, A. Bakrim, J. Carlos, N. Munoz, M. Este´vez, A. Aarab, and R. Lafont (2013). Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. Saudi J. of Biol. Sci., 20: 29–36.

Liu, W. X., J. W. Liu, M. Z. Wu, Y. Li, Y. Zhao and S. R. Li (2009). Accumulation and translocation of toxic heavy metals in winter wheat (*Triticum aestivum* L.): Growing in Agricultural Soil of Zhengzhou, China. Bull Environ Contam. Toxicol., 82: 343-347.

López, M. L., J. R. Peralta-Videa, T. Benitez, M. Duarte-Gardea and J. L. Gardea-Torresdey (2007). Effects of lead, EDTA, and IAA on nutrient uptake by alfalfa plants. J Plant Nutr., 30(8): 1247-1261.

Luptáková, A., D. Gešperová and D. Kupka (2002). The selective chemical - biological precipitation of Cu and Cd from solutions, in.: Fečko, P., Čablík, V. (Eds.) Proceeding from 6th Conference on Environment and Mineral Processing, part II, Ostrava, 509-514.

MacFarlane, G. R. (2003). Chlorophyll a fluorescence as a potential biomarker of zinc stress in the grey mangrove, *Avicennia marina* B., Environ. Contam. Tox., 70: 90-96.

Madyiwa, S., M. J. Chimbari and F. Schutte (2004). Lead and cadmium interactions in *Cynodon nlemfuensis* and sandy soil subjected to treated wastewater application under greenhouse conditions. Physics and Chemistry of the Earth, 29: 1043-1048.

Mcintyre, T. (2003). Phytoremediation of heavy metals from soils. Advanced in Biochemical Engineering/Biotechnology, 78: 98-119.

Mishra, S., S. Srivastava, R. D. Tripathi, R. Govindarajan, S. V. Kuriakose and M. N. V. Prasad (2006). Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol. and Bioch. 44: 25-37.

Muhammad, S. A. A., H. Mumtaz, I. Samina and K. A. Ambreen (2008). Photosynthetic performance of two mung bean (*Vigna radiata*) cultivars under lead and copper stress. International Jour. of Agric. & Biology, 10 (2): 167-172.

Murphy, T. M. (1998). Possible sources of reactive oxygen during the oxidative burst in plants. Kluwer Academic Publishers, pp. 215-246.

Page, A. L. (1982). Methods of Soil Analysis Part 2: Chemical and Microbiological Properties. Soil Sci. Amer. Inc., Madison, Wisconsin, USA.

Poskuta, J. W., E. Parys, E. Romanoska, M. Gadish and B. Worblewska (1988). the effect of lead on photosynthesis C^{14} distribution among photoassimilates and transpiration of maize seedling. In: Quantitative organic microanalysis, Edt. J. and A. Churchill, London.

Prassad, D. D. K. and A. R. K. Prassad (1987). Altered aminolaevulinic acid metabolism by lead and mercury in germinating seedlings of Bajra (*Pennisetum typhoideum*). J. Plant Physiol., 127: 241-249.

Ruley, A. T., N. C. Sharma, S. V. Sahi, S. R. Singh and K. S. Sajwan (2006). Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. Environ. Pollution, 144: 11-18.

Salt, D. E., M. Blaylock, N. P. B. A. Kumar, V. Dushenkov, B. D. Ensley, I. Chet and I. Raskin (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Nature Biotechnology 13: 468-474.

Saradhi, A. P. P. (1991). Proline accumulation under heavy metal stress, J. Plant Physiol., 138: 554-558.

Sayed, S. A. (1999). Effects of lead and kinetin on the growth and some physiological components of safflower. Plant Growth Regulation, 29:167-74.

Selim, A. H. (2000). Studies on the effect of lead and some agents for remediation of lead ions on the performance of carrot plant (*Daliclis carrota* L.). J. agric. Tropic. and Subtropics, 71: 103-118.

Sengar, R. S. and M. Pandey (1996). Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments. Biol. Plant., 38: 459-462.

Sharma, P. and R. S. Dubey (2005). Lead toxicity in plants, Braz. J. Plant Physiol. 17: 35-52.

Sharma, N. C., S. V. Sahi and J. C. Jain (2006). *Sesbania drummondii* cell cultures: ICP-MS determination of the accumulation of Pb and Cu. Microchemical Journal, 81: 163– 169.

Shelmerdine, A., C. Black, S. McGrath and S. Young (2009). Modelling phytoremediation by the hyperaccumulating fern, *Pteris vittata*, of soils historically contaminated with arsenic. Environmental Pollution, 157: 1589-1596.

Shigeoka, S., T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta and K. Yoshimura (2002). Regulation and function of ascorbate peroxidase isoenzymes, J. Exp. Bot., 53: 1305–1319.

Singh, R. P. and M. Agrawal (2007). Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. Chemosphere, 67 (11): 2229-2240.

Singh, R. P., R. D. Tripathi, S. K. Sinha, R. Maheshwari and H. S. Srivastava (1997). Response of higher plants to lead contaminated environment, Chemosphere 34: 2467-2493.

Succuro, J. S. (2010). The Effectiveness of Using *Typha latifolia* (Broadleaf Cattail) for Phytoremediation of Increased Levels of Lead-Contamination in Soil. M.Sc. Thesis, the Graduate Faculty of the University of Akron.

Taylor, L. P. and E. Grotewold (2005). Flavonoids as developmental regulators. Curr. Opin. Plant Biol., 8: 317-323.

Van Den Bussche, F. and D. Van Der Straeten (2004). Shaping the shoot: a circuitry that integrates multiple signals. Trends Plant Sci., 9: 499-506.

Verma, S. and R. S. Dubey (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science, 164: 645-655.

Walter, I. and G. Cuevas (1999). Chemical fraction of heavy metals in a soil amended with repeated sewage sludge application. The sci. total Environ., 226: 113-11.

Wang, C., J. Lu, S. Zhang, P. F. Wang, J. Hou and J. Qian (2011). Effects of Pb stress on nutrient uptake and secondary metabolism in submerged macrophyte *Vallisneria natans*. Ecotoxicol. Environ. Saf., 1016: 1-7.

Wasfy, W., Shindy and E. S. Orrin (1974). Identification of plant hormones from cotton ovules. Plant Physiology 55: 250 – 554.

Welch, R. M., W. A. Norvell, S. C. Schaefer, J. E. Shaff and L.V. Kochian (1993). Induction of iron (III) and copper (II) reduction in pea *(Pisum sativum* L.) roots by Fe and Cu status: does the root-cell plasmalemma Fe (III)-chelate reductase perform a general role in regulating cation uptake. Planta, 190: 555-561.

Wettstein, D. (1957). Chlorophyll totale under submikroskopische form wechsel der plastiden. Exptl. Cell. Res., 12: 427-433.

Xiao shu, Y. LiYan, Z. QuanFa and W. WeiBo (2012). Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. Environ. Sc. and Pollution Res., 19 (3): 893-902.

Xiong, Z. T. (1997). Bioaccmulation and physiological effects of excess lead roadside pioneer species *Sonchus oleraceus*, Environ. Pollut. 97: 275-279.

Yongsheng, W., Q. Liang and Q. Tang (2011). Effect of Pb on growth, accumulation and quality component of tea plant. Procedia Engineering, 18: 214 – 219.

الإصحاح البيئي للتربة الملوثة بالرصاص بواسطة النباتات الشرهة لإمتصاصة

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الملخص العربي:

أجريت تجارب أصص بمزرعة كلية الزراعة بشبين الكوم جامعة المنوفية خلال صيف موسمي ٢٠٠٩ ، ٢٠١٠ لدراسة معالجة التربة الملوثه بالمعادن الثقيلة بواسطة زراعة نباتات شرهه لإمتصاص تلك المعادن مثل عباد الشمس و اللوبيا و عرف الديك حيث أستخدمت سبعة مستويات من معدن الرصاص هي ٠ (كنترول) و ٥٠ و ١٠٠ و ٢٠٠ و ٤٠٠ و ٨٠٠ و ١٦٠٠ مللجرام / كجرام تربة. وخلال تجميع ذلك المعدن بواسطة النباتات تحت الدراسة من التربة الملوثة تم دراسة صفات النمو الخضري و بعض من الصفات الفسيولوجية و الكيميائية مثل صبغات البناء الضوئي و العلاقات المائية و الكربوهيدرات الكلية و السكريات الكلية و تركيز البرولين و نشاط بعض الإنزيمات و البروتين الكلي و محتوي النبات لبعض من العناصر المعدنية و تركيز الهرمونات النباتية الداخلية والتركيب التشريحي لتلك النباتات وبعض الصفات الكمية والنوعية للمحصول. أدي تجميع معدن الرصاص داخل المحاصيل المختلفة عند جميع مستويات التلوث بالرصاص إلي نقص في صفات النمو الخضري ، محتوي الماء الكلي و النسبي ، معدل النتح ،الصبغات النباتية ، نشاط إنزيم الفينول أوكسيديز ، تركيز النيتروجين والفسفور والبوتاسيوم ، تركيز الهرمونات النباتية الداخلية ، ونقص المحصول و مكوناته لكل من المحاصيل تحت الدراسة. بينما أدي إلي زيادة نقص المحتوي المائي للورقة ، الضغط الإسموزي وزيادة تركيز البرولين و نشاط إنزيم البيروكسيديز ، تركيز معدن الرصاص داخل الأعضاء النباتية المختلفة. ومما سبق يتضح أن ، إستخدام النباتات الشرهة الإمتصاص للمعادن الثقيلة أدي إلي نقص في تركيز المعدن الثقيل الرصاص في التربة الملوثة به وكان أفضل المحاصيل المستخدمة لهذا الغرض هو عرف الديك يليه عباد الشمس ثم اللوبيا.