PHYTOREMEDIATION OF A Pb-POLLUTED SOIL USING MUSTARD PLANT SPECIES

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ABSTRACT:

A greenhouse pot experiment was conducted to clarify the ability of both White mustard (*Sinapis alba* L.) and Indian mustard (*Brassica juncos* L.) as a phytoremediation for a polluted Pb-soil. Plants were grown on a soil treated with Pb at rates 154, 450, 750, and 950 mg kg^{-1} soil. Soil pots were daily irrigated with tap water. The plants allowed to growing for four months. One week before harvest, ethylene-diamine-tetraacetic acid (EDTA) as a chelated agent was applied at a rate of 0.5 g kg^{-1} soil for 50 % of the experimental pots. Either EDTA-treated or untreated plants were harvested 127 days after planting.

The obtained results showed that white mustard and Indian mustard plants grown in Pb contaminated soil can tolerate and accumulate significant amounts of lead (Pb) in their roots and shoots. The results suggest that adding EDTA can promote the bioavailability of Pb in the soil and increase the propensity for Pb-uptake by plants into roots and shoots. It could be concluded that White mustard and Indian mustard can be grown under elevated Pb conditions due to their suitability as a potential crop species for phytoremediation of polluted soils with heavy metals.

Keywords: Lead, phytoremediation, phytoextraction, EDTA chelate.

INTRODUCTION:

Besides being an economical, energy efficient and environmental friendly method, phytoremediation could be applied to large areas and is useful for solving a wide variety of contaminants (metal, radionuclide and organic substances). Phytoremediation could be specified into many applications **(Cunningham *et al*., 1995**) including: phytoextraction, in which plants decontaminate soil through uptake of heavy metals into aerial part and then can be harvested and removed from the site; Phytostabilization, in which plants are used to minimize heavy metal mobility in contaminated soil; and Phytovolatilization, in which plants extract volatile metals from soil and volatilize them from foliage.

Adding chelators might provide a more effective approach for enhancing phytoremediation. **Bricker *et al*. (2001)** found that the EDTA treatments increased solubilizing soil Pb and Cd for roots uptake. Soil Cd was, generally, more mobile for plant uptake than soil Pb. Adding EDTA at rate of 0.2 % was most successful in promoting Pb and Cd uptake by both maize and mustard. Although Pb concentrations were lower for maize than mustard, the former removed more total Pb (0.2 mg per pot), as compared to mustard (0.03 mg), due to its higher biomass production.

Chen and Cutright (2001) investigated the ability of EDTA for enhancing the phytoremediation of Cd, Cr, and Ni from contaminated soil.Uptake into and translocation within sunflower (*Helianthus annuus* L.) was determined. They found that EDTA at a rate of 0.5 g kg^{-1} significantly increased the shoot concentrations of Cd and Ni from 34 and 15 to 115 and 117 mg kg^{-1}, respectively. The total removal efficiency for EDTA was 59 µg $plant^{-1}$. **Turgut *et al*. (2004)** studied the effect of two rate of EDTA on phytoremediation of Cd, Cr, and Ni from soil using two cultivars sunflower (*Helianthus annuus* L.) EDTA at a concentration of 0.1 g kg^{-1} yielded the best results for both cultivars achieving a total metal uptake of 0.73 mg/kg as compared to 0.40 mg/kg when EDTA was added at 0.3 g kg^{-1}.

Wu *et al.* (2004) found that the addition of EDTA (disodium salt, 3 m mol_c kg^{-1}) to pots of a paddy soil significantly enhanced the mobilities of soil Cu and Pb but not Zn and Cd. EDTA increased shoot Cu and Pb concentrations in Indian mustard plants growing in the soil. EDTA addition led to elevated soil solution concentrations of Cu, Zn, Pb and Cd for about 1 month. Rainfall after EDTA application, as simulated by column leaching experiment, increased the concentrations of Cu, Zn, Pb and Cd linearly in leachate with increasing EDTA dosage from 0 to12 m mol_c kg^{-1}. About 68 % of the added EDTA tended to chelate soil Cu, Zn, Pb and Cd, and 32 % was chelated with other leached ions (**Roongtanakiat, 2009).**

The present study was conducted to study the ability of both white mustard and Indian mustard as phytoremediation for a polluted Pb-soil. The work also clarifies the effect of adding EDTA at two rates on phytoremediation of Pb contaminated soil.

MATERIALS AND METHODS:

A greenhouse pot experiment was carried out in greenhouse at Soil, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt. Two phytoremediation plants, *i.e.*, Indian mustard (*Brassica juncos* L.) and White mustard (*Sinapis alba* L.) were used. Fifteen seeds of either Indian mustard or white mustard per each pot were sown, each pot containing 8 kg Pb-polluted soil collected from El-Gabal El-Asfar. The current experiment comprises four treatments of artificially contaminated soils, with total Pb contents of:

1. Control 154 mg Pb/kg soil (Pb0).
2. 450 mg kg^{-1} soil (Pb1).
3. 750 mg kg^{-1} soil (Pb2).
4. 950 mg kg^{-1} soil (Pb3).

The corresponding available contents were of 22.35, 95.57, 241.91, and 318.4 mg Pb/kg soil, respectively.

Pots were daily irrigated with tap water for one week before seed planting for stabilizing soil material within the experimental pots. After that, seeds of both plants of Indian mustard (*Brassica juncos* L.) and White mustard (*Sinapis alba L.*) were sown, and then all soil pots were daily irrigated with tap water. The plants were allowed to grow for four months. One week before harvesting, half of the experimental pots were used for adding EDTA solution as chelators at a rate of 0.5 g EDTA per kg dry soil as recommended by **Robinson *et al.* (1999)**, and then plants were allowed to continued grow. At the end of the experiment (127 days after planting), soil samples were taken and plants were removed from all pots (first group without

EDTA and second one with EDTA), cleaned, washed, oven-dried at 60 ºC, and then kept for the studied analyses.

Chemical and physical properties of the experimental soil in initial state are presented in Table 1.

Table 1. Some chemical and physical properties of the studied experimental soil.

Soil character	Value	Soil character	Value
Particle size distribution %:		*Total heavy metals (mg kg^{-1} soil):*	
Sand	68.7	Fe	15500
Silt	8.8	Mn	270.0
Clay %	22.5	Zn	561.5
Texture class	SCL*	Cu	96.0
Soil pH (1:2.5 soil water suspension)	6.11	Cd	3.0
Soil salinity, EC (dSm^{-1})	1.32	Co	4.20
Organic matter %	6.15	Ni	28.0
$CaCO_3$ %	0.57	Pb	154.5
CEC (c mol kg^{-1} soil)	19.42		
Soluble cations and anions (meq/L):		*Available heavy metals (mg kg^{-1} soil):*	
Ca^{++}	5.00	Fe	100.40
Mg^{++}	4.00	Mn	57.80
Na^{+}	3.23	Zn	96.50
K^{+}	0.70	Cu	34.00
CO_3^{--}	0.00	Cd	0.03
HCO_3^{-}	4.00	Co	0.50
Cl^{-}	3.00	Ni	4.05
SO_4^{--}	5.93	Pb	22.35

*Sandy clay loam

Particle size distribution was determined using the International Pipette method as described **by Piper (1950)**. Organic matter content was determined according to the method of **Walkley and Black (1982)**. Calcium carbonate percentage was determined volumetrically, using Collin's Calcimeter, Soil pH was measured in soil water suspension 1:2.5 using a pH meter **(Jackson, 1973)**. Electrical conductivity (ECe) and soluble ions in the soil paste extract were determined as described by **Black *et al.* (1982)**. Cation exchangeable capacity (CEC) was determined using the ammonium acetate solution according to **Bower *et al.* (1952)**.

Heavy metal contents of plant samples were determined according to method of **Chapman and Pratt (1961)**. Available heavy metal contents of soil samples were extracted with ammonium bicarbonate-DTPA (AB-DTPA) according to method of **Lindsay and Norvell (1978)**, while total metal contents were digested by aquaregia according to method of **Cottenie *et al.* (1993)**. Total and available contents of heavy metals in either extracts or digested solutions were measured by using Inductively Coupled Spectrophotometer Plasma.

RESULTS AND DISCUSSION:

I. *Lead contents in shoots and roots of the tested plants:*

Data of Pb contents in white mustard (*Sinapis alba* L.**)** and Indian mustard (*Brassica juncea* L.) plants are presented in Table 2.

Table 2. Lead contents in white mustard (*Sinapis alba*) and Indian mustard (*Brassica juncea*) under soil Pb-treatments at harvest.

Pb treatment	Pb contents as mg kg^{-1} plant tissues					
	White mustard			Indian mustard		
	Shoots	Roots	Seeds	Shoots	Roots	Seeds
Control (Pb0)	9.08	10.45	0.61	10.25	11.84	0.72
Pb1	64.67	95.74	4.19	99.82	142.10	5.97
Pb2	88.16	136.85	7.17	140.75	178.20	9.04
Pb3	115.21	178.65	8.03	186.90	237.00	9.87

Pb contents in shoots and roots of *Sinapis alba* reached about 7, 10, and 13 folds as found at the control treatment (soil initial state) for the applied Pb contents for treatments Pb1, Pb2, and Pb3, respectively. Also, the obtained data in Table 2 showed that Indian mustard plants were more effective for Pb removed from the treated soils. As Pb contents in Indian mustard shoots were about 10, 14 and 18 folds as found at the control treatment for 450 (Pb1), 750 (Pb2), and 950 (Pb3) mg kg^{-1} soil, respectively. The same trend was observed for seed contents of Pb in both tested plants. The data showed that the average accumulation of lead in roots is 60% and 67% of the total accumulate Pb for White mustard and Indian mustard, respectively.

It was observed that during the duration of this study, the root systems of the two tested plants were extensive similar to the fibrous root systems of most monocots (wheat), so such effective root systems resulted in relatively high Pb contents in both roots of White mustard (*Sinapis alba*) and Indian mustard (*Brassica juncea* L.) as compared to its contents in shoots for the two tested plants. who found that roots depth and density (plant species) are important factors in phytoextraction. This is in agreement with **(Kabatta-Pendias and Pendias, 1992)**. (**Begonia *et al*. (2002)**.

The superiority of Indian mustard (*Brassica juncea*) for removing Pb from polluted soil is confirmed by some works who found that such plant species was an effective one in lead remediation, because of its great biomass production, but it accumulated 95% of lead in its roots **(Begonia *et al.*, 1998)**.

II. *Dry matter weight of plants under lead treatment:*

In general, the lowest value of dry matter weight for shoots of each plant was recorded at the control treatment (Table 3).

Table 3. Dry matter weights of White and Indian plant organs as affected by applied soil Pb-contents at harvest.

Pb treatment	**Dry matter weight (g pot^{-1})**					
	White mustard			**Indian mustard**		
	Shoots	Roots	Seeds	Shoots	Roots	Seeds
Control (Pb0)	8.54	3.06	1.65	9.70	3.51	2.06
Pb1	13.70	2.81	1.50	15.19	2.95	1.86
Pb2	17.81	2.50	1.35	19.61	2.60	1.51
Pb3	22.71	1.93	0.91	24.90	2.40	1.21

Meanwhile, the highest value, however, was recorded at the plants received the highest rate of Pb3 (950 mg kg^{-1} soil). On the contrary, values of root and seed dry matter weights were gradually decreased with increasing the applied Pb rates as compared with the control treatment. Thus, it could be concluded that increasing Pb-contents in soil might be increment dry matter weight of shoots (higher biomass) for both *Sinapis alba* and *Brassica juncea* species.

Actually, plant dry matter weight can be used as an indicator for the overall health of either White mustard and Indian mustard plants. The stimulation of plant growth treated by Pb could be attributed with an increase in the synthesis of cell wall polysaccharides resulting from Pb exposure. The shoot biomass increased by about 60, 108 and 160% for white mustard and 56,102 and 156.7% for Indian mustard for treatment Pb1, Pb2, and Pb3, respectively.

III. *Lead content in soil*:

The obtained data (Table 4) showed that Pb contents in the treated soils increased as applied Pb-rates increased, where the highest value was found in the soil treated with highest Pb-rate (950 mg kg^{-1} soil).

Table 4. Soil content of total and available Pb at applied different rates under cultivation with the testes two plants at harvest.

Pb treatment	Pb content as mg kg-1 soil			
	White mustard		Indian mustard	
	Total	Available	Total	Available
Control (Pb0)	134.13	13.21	123.30	9.50
Pb1	311.50	90.57	286.67	77.83
Pb2	557.67	236.80	472.75	197.55
Pb3	801.87	310.60	789.33	261.40

Meanwhile, the lowest value was recorded at the untreated soil with Pb (the control treatment) under cultivation of both *Sinapis alba* and *Brassica juncea* species. The available soil Pb-contents showed also a parallel increase with increasing the applied Pb-rate, Available soil Pb- content reached the greatest value at the highest rate of 950 mg kg^{-1} soil.

IV. ***Effect of EDTA on phytoremediation of Pb:***

a. Soil:

Results in Table 5 showed that EDTA increased soil availability and mobility of Pb in the soil with increasing the applied Pb contents (450, 750, 950 mg kg^{-1} soil), This was companied with increasing Pb-uptake by plant organs and parallel decreasing in either total contents or available fractions of Pb metal.

Table 5. Soil content of total and available Pb at applied different rates under applied EDTA and cultivation with the testes two plants at harvest.

Pb treatment	Pb content as mg kg-1 soil			
	White mustard		Indian mustard	
	Total	Available	Total	Available
Control (Pb0)	110.20	8.52	101.17	6.35
Pb1	258.43	58.18	238.67	51.90
Pb2	460.17	152.25	390.67	131.82
Pb3	61.73	192.45	658.67	173.50

The obtained data in Table 6 showed that there were pronounced decreases in both total and available fractions of Pb of the soils amended with EDTA as compared to those contents in the untreated ones under either *Sinapis alba* or *Brassica juncea* species. The relative decrease percentages reached about 20 % for total Pb-contents in case of the tested two plants. The corresponding reduction in the available fractions of Pb was much higher, where the relative decrease percentages reached about 55 % in case of *Sinapis alba* vs about 50 % in case of *Brassica juncea* species.

It is noteworthy to mention that the tested grown plants at different Pb-contents had no visible symptoms of Pb toxicity before adding EDTA. However, 2-4 days after applied EDTA treatment, numerous brown dots on the leaves were appeared and the whole leaf became yellow, indicating phytotoxicity causes. That means that EDTA increased Pb-removal from soil, probably due to increasing the mobility of soil Pb and concentrated it in shoots of both plant species.

b. Plant:

The obtained data presented in Figs. (1 & 2) showed that the different organs (shoots, roots, seeds) of either *Sinapis alba* or *Brassica juncea* species grown in EDTA treated soil attained more amounts of Pb-contents as compared with those EDTA-untreated soils. The addition of EDTA at rate of 0.5 g/ Kg enhanced Pb desorption from soil to soil solution and facilitated transport from roots to shoots.

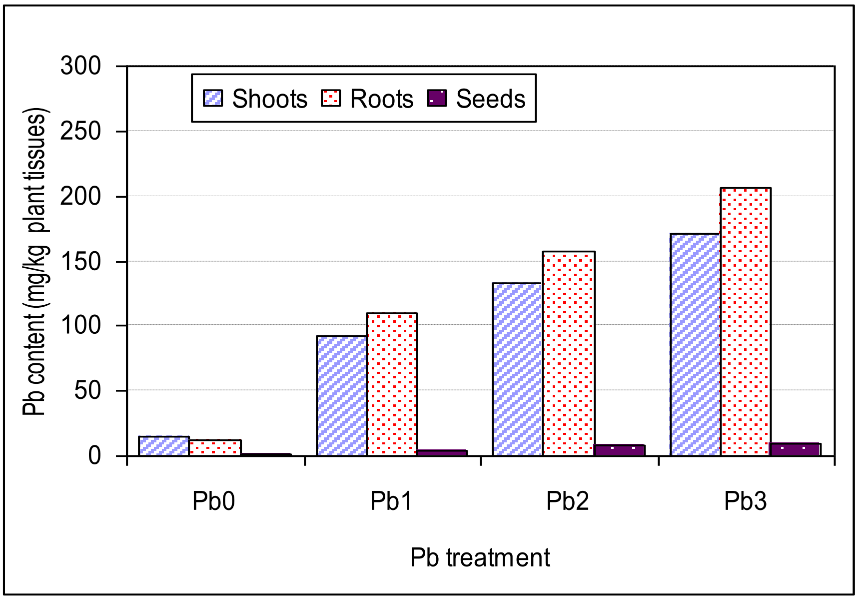

Fig. (1): Effect of Pb treatment on Pb content of White mustard plant tissues.

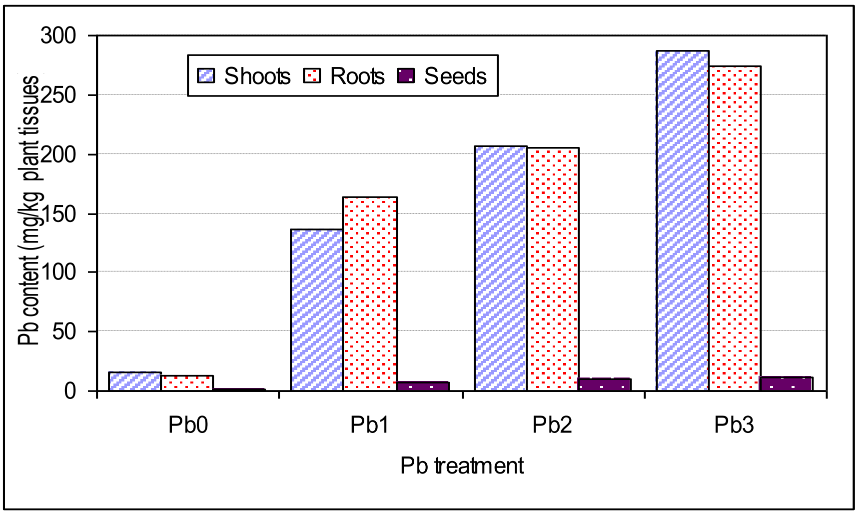

Fig. (2): Effect of Pb treatment on Pb content of Indian mustard plant tissues.

V. *Dry matter weight f plants under EDTA treatment:*

Results of dry matter weights of shoots, roots and seeds of both plants of *Sinapis alba* and *Brassica juncea* species under applied Pb treatments are shown in Table 6. In general, the highest values of shoot dry matter weight were recorded at the treatment of highest Pb-rate of 950 mg kg^{-1} soil as compared with the control treatment. Adding EDTA resulted in pronounced increases in the dry matter weight of shoots by about 60,109 and167% for white mustard and by 55, 99 and 155% for Indian mustard for Pb1, Pb2 and Pb3, respectively. On the contraru, the dry matter weight of both root and seed took place an opposite trend, where their value tended to decrease with increasing the applied Pb-content in the polluted soils as compared with the control treatment. The decrease in roots dry weight was 7-36% and 16-31%for white mustard and Indian mustard, respectively. He decrease in seeds dry weight was 10-45% and 10-41% for white mustard and Indian mustard , respectively.

Table 6. Dry matter weights of White and Indian plant organs as affected by applied soil Pb-contents and EDTA as amended agent at harvest.

Pb treatment	Dry matter weight (g pot-1)					
	White mustard			Indian mustard		
	Shoots	Roots	Seeds	Shoots	Roots	Seeds
Control (Pb0)	6.78	2.43	1.33	8.10	2.91	1.71
Pb1	10.90	2.24	1.20	12.50	2.45	1.54
Pb2	14.18	2.00	1.07	16.15	2.16	1.25
Pb3	18.15	1.54	0.73	20.70	2.00	1.00

Finally, phytoextraction is gaining acceptance as a cost-effective and environmentally friendly phytoremediation strategy for reducing toxic metal levels from contaminated soils. Also, from this study it is found that white mustard (*Sinapis alba* L.) and Indian mustard (*Brassica juncea* L.) plants can tolerate and accumulate significant amounts of lead (Pb) in its roots and shoots when grown in a contaminated soil with Pb. The addition of ethylene-diamine-tetraacetic acid (EDTA) further enhanced the shoot uptake of Pb.

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المعالجة النباتية لتربة ملوثة بالرصاص باستخدام أصناف من نباتات الخردل

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أجريت تجربة أصص خضريه لتوضيح مدى قابليه و قدرة كل من نباتي الخردل الأبيض والهندى White mustard (*Sinapis alba* L.) and Indian mustard (*Brassica juncea* L.) على المعالجه النباتيه لتربه ملوثه بالرصاص، حيث زرعت تلك النباتات فى تربة ملوثه بمستويات مختلفة من الرصاص Pb0, Pb1, Pb2 and Pb3 (١٥٤، ٤٥٠، ٧٥٠، ٩٥٠ مجم رصاص /كجم تربة). وقد تم رى النباتات يوميا على إمتداد فترة نمو النباتات حتى مدة ١٢٠ يوم من الزراعة، وقبل حصاد النباتات بأسبوع تم معالجه تربه ٥٠٪ من أصص التجربة بمركب مخلبى من الـ EDTA (إيثلين داى أمين تترا أستك أسيد) بمعدل ٠.٥ جم/كجم تربة، ثم تم حصاد نباتات التجربة جميعها (المعاملة وغير المعاملة بـEDTA) بعد ١٢٧ يوما من الزراعة، وقد تم تجهيزها للتقديرات المطلوبة.

وتشير النتائج المتحصل عليها من الدراسه إلى أن نباتى الخردل الابيض والهندى لهما القدرة على مقاومة التلوث وإستخلاص وتجميع عنصر الرصاص من التربه بكميات معنويه فى عضوى النبات (السيقان والجذور) عند زراعتهما فى تربه ملوثه بالرصاص. كما توضح نتائج الدراسة أن إضافة المركب المخلبى (EDTA) كمحسن للتربة تعتبر مفيدة من ناحية التيسر البيولوجى للرصاص فى التربه مع زياده موازية لإمتصاصه بواسطة النباتات تحت الدراسة فى كلا أجزائها (الجذور والسيقان). ومن نتائج هذه الدراسة يمكن إستنتاج ان كلا نباتى الخردل الأبيض والهندى يمكنهما النمو تحت ظروف التلوث بعنصر الرصاص لصلاحيتهما كحاصلات لها القدرة على المعالجة النباتية للاراضى الملوثة بالعناصر الثقيلة.