Effect of Synthetic Chelators and Low Molecular Weight Organic Acid in Enhancing Uptake of Lead and Nickel by Mustard (*Sinapis Alba L.*)

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The aim of this study is to evaluate the effect of ethylene diamine tetra acetic acid disodium salt (EDTA), diethylene triamine pentaacetic acid (DTPA) and citric acid (CA) applied separately to the soil sample on enhancement of lead (Pb) and nickel (Ni) uptake by mustard (*Sinapis alba L.*) in greenhouse experiment. Data indicated that application of different chelators led to significant increase in heavy metals uptake with decrease in weight of dry matter production of used plant compared to control treatment. The type of chelators and their rates of application were the most effective factors controlled heavy metals uptake by plants. Results also indicated that Application of DTPA and EDTA at highest concentration, led to significant increase in shoot Pb concentration regardless the incubation time, the same trend was also observed for Ni. In addition, accumulation of Pb and Ni in the tissues corresponded to the concentrations of Pb and Ni extracted from the used soil. Our work emphasized that the accumulation of metal in the shoots of *Sinapis alba* could be enhanced through the application of synthetic chelators to the soil system, facilitating high biomass accumulation as well as metal uptake.

Keywords: Mustard plant, Heavy metal, Citric acid, Chelators.

Lead (Pb) and nickel (Ni) are the most toxic heavy metals in the environment at high concentrations. Human activities such as mining, smelting, dumping of municipal such as composts and sewage sludge, manufacturing processes and disposal of used batteries, all are the main sources of these pollutants. Some other sources such as fertilizers or pesticides may be supply significant amounts of such pollutants on agricultural land (Schmidt and Sticher, 1991). Although Ni is not toxic elements in metabolic processes of plants or animals and Pb in some cases, however, increasing the accumulation of these metals to high levels may led to depressing plant growth and become toxic source.

Recently, there is considerable interest in the development of low-cost techniques for remediation of sites contaminated with Pb and Ni, these
techniques must have minimal environmental side effects (Jung and Thornton, 1997). Phytoremediation considered one of such techniques.

Phytoremediation, as defined by Dietz and Schnoor (2001) is the use of vegetation for the in situ treatment of contaminated soils, sediments, and/or ground water. Phytoremediation (and especially phytoextraction) has recently been proposed as an effective method to remediate soils contaminated with Pb and Ni (Lock and Janssen, 2001). The efficiency of phytoremediation depends on several factors, for example, plant must produce sufficient biomass while accumulating a high concentration of the certain metal. The metal-accumulating plants also need to be responsive to agricultural practices to allow repeated planting and harvesting of the metal-rich tissues (Blaylock et al., 1997). Therefore, enhancing metal accumulation in existing high yielding crop plants without diminishing their yield is the most feasible strategy in the development of phytoremediation (Bricker et al., 2001). Phytoextraction is an environmental friendly and cost-effective approach that uses green plants for the in situ risk reduction for contaminated soil, sludge, sediments, and groundwater through contaminant removal. This process has been investigated in several field experiments (Kayser et al., 2000). There are two main strategies: the use of hyperaccumulator plant species that typically have relatively low yields and use of non-hyperaccumulator species with higher yields but lower metal uptake capacities. The criterion for hyper accumulation varies for different metals, but was summarized by Baker et al. (1991) and (1994) for Pb and Ni as >1000 mg kg⁻¹ of shoot dry matter.

The bioavailability of heavy metals is an important factor in the process of phytoextraction by non-hyper accumulators, especially in neutral or calcareous soils. There are two main approaches that have been used to increase the bioavailability of heavy metals in soils: lowering soil pH and adding synthetic chelates (Blaylock et al., 1997). There have been numerous studies focusing on one of these two approaches but few have deal with the combined use of both approaches (Kayser et al., 2000). Ethylene diamine tetra acetic acid (EDTA) is an effective chelated material that has been found to increase the solubility of heavy metals in soil and their bioavailability to plants, and the effect can be improved by decreasing soil pH.

The aim of this paper is to evaluate the performance of the naturally occurring biodegradable chelator citric acid (CA) and synthetic chelators EDTA and DTPA for solubility and uptake of heavy metals in contaminated soil by mustard (Sinapis alba L.)

Material and Methods

Soil characteristics

Soil sample (0-30 cm) was collected from the Farm of Faculty of Agriculture, Cairo University, Egypt. By using the standard methods, selected soil sample is characterized by pH (7.6), EC (1.80 dSm⁻¹), OM (1.7%), total CaCO₃ (1.5%) and CEC (33 meq/100mg). The selected soils sample has 14.5 % coarse sand, Egypt. J. Soil Sci. 49, No. 2 (2009)
31% fine sand, 22% silt and 32.5 clay, the texture of this soil is silt clay loam. For total and available Pb and Ni, soil analysis showed that these values were 30, 2.5 ppm for Pb and 1.2, 1.1 ppm for Ni, respectively. Soil sample was air-dried, grounded, and passed through a 2-mm sieve for pot experiments in a greenhouse.

**Experimental design and treatments**

Two kilograms of air-dried soil was weighed and transferred into a plastic pot 10 cm in diameter. The soil samples were spiked with 200 mg kg⁻¹ soil of Pb or Ni in nitrate form represents a toxic level of Pb in soil (Zaghloul and Abo-Seeda, 2003) and mixing thoroughly. The moisture content was adjusted to about 70% of total water holding capacity. The vaporized or absorbed water was replenished every 2 days. The spiked soils were kept placed in a greenhouse for 1 month to allow it to reach the maximum equilibration. Five seeds mustard (Sinapis alba L.) were sown directly onto the soil and thinned to two plants per pot after 7 days of germination.

Pots were fertilized with 50 mg N (as NH₄NO₃), 100 mg P (as calcium super phosphate) and 40 mg K (as potassium sulfate) per kg soil before seeding micronutrients. Iron applied as FeSO₄.7H₂O, Mn as MnCl₂.4H₂O, B as H₃BO₃, and Mo as Na₂MoO₄.2H₂O were also applied at concentrations of 2, 4, 1, and 0.2 mg kg⁻¹, respectively.

Three weeks after thinned, three different chelators EDTA, DTPA and CA were applied separately at three dosages 2, 4 and 6 mmol kg⁻¹ soil for each chelator to the soil samples in solution form. Treatments were performed in triplicate in a completely randomized design.

After 30 days of chelators applied to soil, plant shoots were harvested at the soil surface. At harvest, the growing plants were in the growth stage without flowering, in other words no seeds were generated. The soil was then broken up and roots were collected by hand. The roots were washed in tap water until cleaned from soil particles. Both shoots and roots were further washed with deionized water, oven-dried at 70°C for 24 hr, weighed, and then ground and passed through a 2.0mm sieve. 0.5 g dried weight of plant powder were digested overnight in 14M HNO₃ (5 ml) and 30% H₂O₂ (v/v; 10 ml) and heated at 120°C for 2 hr (Mench et al., 1994). The digested solutions were filtered using Whatman No. 42 filter paper and diluted to 50 ml with distilled water.

The soil samples which were taken after 15 days (soil without plant to represent the rhizosphere effect) and 30 days from time of applied chelators, both were sieved through a 2-mm sieve and extracted by DTPA to their content of Pb and Ni by adding 0.005 M DTPA, 0.01M CaCl₂ and 0.1 M triethanolamine (pH 7.3) to give a 1:2 (w/v) soil:solution ratio (Mi.P.A.F., 2000). The total and extractable Pb and Ni were determined by atomic absorption spectrophotometry (Perken Elmer – 2380). The total amounts of heavy metals were determined by soil digestion in a mixture of HF–HNO₃–HClO₄–H₂SO₄ (Baker and Amacher, 1982).

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Dry matter production

Barrier to discuss the obtained results, it should be mention that visual symptoms of toxicity such as some chlorosis and necrosis on leaves, particularly in old ones, were observed in the EDTA and DTPA treatments especially at high levels applied after 30 days.

Data in Table 1 represents shoot (SDW) and root (RDW) dry weight production of mustard for different treatments applied. In SDW, compared to control, data indicated that in the treatment of any chelator applied to Pb or Ni contaminated soils, led to significantly reduction in dry matter production of this organ with exception observed in citric acid treatment applied at the lowest concentration (2 mmol kg⁻¹). However, the largest reduction in dry matter was observed in DTPA treatment. With respect of reduction effect of such materials, the high reduction observed in plant growth for EDTA compared to other treatments may due to the toxicity of EDTA itself and its metal complexes (Chen and Cutright, 2001). Wu et al. (2006) was also demonstrated that the leaves of B. juncea developed numerous brown dots after 2–4 days of adding 3.0 mmol kg⁻¹ EDTA to the soil. Thus, high biomass production by plants treated with less phytotoxic chelates could be a key factor for removal of some metals from soil.

In RDW regardless the type of chelators applied and compared to the control treatment, addition of these materials to Pb or Ni contaminated soils, led to significant (p < 0.05) reduction in this plant parameter, with an exception observed for Ni contaminated soil treated with 2 mmol kg⁻¹ citric acid (Table 2). Moreover, the root dry matter decreased with increasing the rate of application of these materials. The comparison between SDW and RDW, results indicated that for each chelator, the dry matter production of shoots were much higher than those of roots. In addition, increasing the application rate of chelators to 6 mmol kg⁻¹, led to decrease shoot or root dry weight compared to 2 mmol kg⁻¹ application rate.

**TABLE 1.** Dry matter yield (g pot⁻¹) in the shoot and root of mustard grown in contaminated soil and treated with different chelators.

<table>
<thead>
<tr>
<th>Element</th>
<th>Organ</th>
<th>Cont.</th>
<th>EDTA</th>
<th>DTPA</th>
<th>CA</th>
<th>LSD 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pb</td>
<td>Shoot</td>
<td>6.16</td>
<td>4.4</td>
<td>4.20</td>
<td>3.78</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>3.08</td>
<td>1.8</td>
<td>1.61</td>
<td>1.47</td>
<td>1.82</td>
</tr>
<tr>
<td>Ni</td>
<td>Shoot</td>
<td>5.60</td>
<td>4.2</td>
<td>3.78</td>
<td>3.50</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>3.1</td>
<td>1.8</td>
<td>1.68</td>
<td>1.54</td>
<td>1.40</td>
</tr>
</tbody>
</table>

Concerning different pollutants applied in relation to their response in uptake according to different chelators applied and the presences of these pollutants in plant organs, data showed that EDTA, DTPA and CA treatments applied at 6 mmol kg⁻¹ soil, for example, significantly decreased SDW of mustard plants in soil contaminated with Pb by about 39%, 35% and 32 % respectively, while the

same values were 38%, 34 and 29% in Ni polluted soil. Under the same conditions, RDW, decreased significantly by about 52%, 48% and 46%, while the same values were 50%, 50 and 29% respectively, in soil polluted with Ni.

Effects of chelators on Pb and Ni concentrations and uptake by mustard plant

Data illustrated in Fig. 1 and 2 indicated that Pb and Ni concentrations and uptake by shoots and roots were significantly increased over control treatment by several folds; this trend was observed in all chelators applied and at any concentration added. Also, the effects of used chelators on Pb and Ni uptake were varied according to type and rate of application of chelators. For example, depending on concentration of chelators applied, data indicated that heavy metals accumulations in shoots were ranged between 0.1 and 0.4% of dry weight. Concerning the effect of type of chelators used, it was noticed that Pb uptake in mustard take the increasing order EDTA > DTPA > CA, indicating that EDTA was the most effective materials tested in Pb Phytoextraction. In this respect, Pb concentration in shoot was increased by about 9 folds over control at 6 mmol EDTA applied to soil sample. Epstein et al. (1999) reported that in Indian mustard the concentration of Pb was increased by about 100-folds in shoots after EDTA application to used soils.

For Ni pollutant, data presented in Fig. 2 indicated that increasing the application rates of chelator led to increase the concentration and uptake of Ni by mustard plant. In addition, citric acid was the most effective chelator material applied among other materials used to enhance phytoextraction. The Ni concentration in shoot was increased by about 6.6 folds in soil treated with 6 mmole citric acid compared to control treatment. Terry and Banuelos (2000) suggested that citric acid could be involved in Ni transport within cucumber plants. Coincidently, data illustrated in Fig. 2 indicated that citric acid was highly effective material in transporting of Ni to shoots of mustard.

The supporting action of CA in Ni transport within plants was reported by different authors, Krämer et al. (2000) reported that in Thlaspi goesingense was used as hyperaccumulator plant; Ni was predominantly located in the vacuole as a Ni-organic complex, possibly this action related to the effect of citric acid. It should be mentioned that understanding of the physiological mechanisms underlying accumulation of metals in shoots, however, is still incipient (Kramer, 2005). More information on the processes involved in chelate-induced translocation in plants will shed light on chemically assisted phytoremediation.

Successful removal of metals from contaminated soils must be seen as a combined result of sufficient metal concentrations in shoots and high biomass production rather than by one of these factors in isolation. As highlighted by Ebbs and Kochian (1997), emphasis for phytoremediation should be placed on the total amount of contaminant removed from soil. Thus, higher biomass production can sometimes compensate for lower metal-concentrated shoots. For instance, Fig. 2 also indicates that the high net removal of Ni from soil using CA (1184 ug) may be related to its ability to shuttle Ni up to shoots as previously commented.

Fig. 1. Pb concentration and uptake in mustard as a function of the concentration of different chelators mmol kg\(^{-1}\) applied to soil.

Effect of synthetic chelators and low molecular...
These values were increased after 15 days to 38.4, 56 and 21.1 mg kg⁻¹ by addition 6 mmol kg⁻¹ of EDTA, DTPA and CA, respectively. In Ni contaminated soil the concentration of this pollutant was 10.8 mg kg⁻¹, which increased after 15 days to 50.4, 68.2 and 20.3 mg kg⁻¹ by the addition of 6 mmol kg⁻¹ of EDTA, DTPA and CA, respectively (Fig. 3). Worth to mention that the same conclusion was observed by Greman et al. (2003).

Fig. 3. Effect of different levels of chelants on extracted Pb and Ni in the used soil.

In general, increasing of Pb and Ni uptake by mustard was observed at highest application rate of EDTA, DTPA and CA in the studied soil. At the end of the experiment, the mobility of Pb in the soil at the highest rate of chelates increased to 44.2, 50.7 and 23.8 mg kg⁻¹ with EDTA, DTPA and CA,

respectively (Fig. 3). A major factor responsible for the observed surge of soluble Pb concentration in soil solution may be the complication of Pb by the chelating agents that promotes Pb desorption from soil to extracts (Jung et al., 1997). Barona et al. (2001) pointed out that Pb remained weakly adsorbed to soil components after a chelating solution was applied. Also, a wide variation in the degree of Pb desorption was observed in the soils treated with chelants. The ability of DTPA to solubilize more Pb than other chelates may be attributed to a function of the strength of the bond between a metal and a chelate agent, which is generally stronger for synthetic chelates compared (Martell and Smith, 1974). Ni is not strongly held by clay and Fe-oxihydroxide surfaces relative to transition Pb element (Puls and Bohn, 1988). Consequently, Ni was more mobile than Pb in the studied soil. Thus, Ni in the soil solutions for all treatments during the experimental period was always at detectable levels. The concentrations of Ni in most cases were much higher than those of Pb, particularly in soils to which synthetic chelators EDTA and DTPA were added. Furthermore, the Ni concentrations in the soil solutions treated with EDTA and DTPA were markedly higher than those in the control soil and those treated with citric acid. The mobility of Ni in the soil at the end of the experiment increased to 50.4, 68.2 and 20.3 mg kg⁻¹ with EDTA, DTPA and CA, respectively (Fig. 3).

Our results indicate that the effects of EDTA persist in soils for longer and remain reactive even after harvesting. This effect was more apparent in soils having higher amount of Ni and at the highest rate of EDTA application. The concentration of available Ni was 50.4 mg kg⁻¹ after 15 days 6 mmol kg⁻¹ of EDTA application, which increased to 58.2 mg kg⁻¹ after 30 days and 588 mg kg⁻¹ after 30 days (Fig. 2). Kos and Leštan (2003) suggested that long-lived chelating agents, such as EDTA are inappropriate for use in enhanced phytoextraction; its longevity will cause elevated metal mobility, even after harvesting plants. Hence, although the concentration of mobilized Pb increased with increasing EDTA concentration, application of higher dose of EDTA to Pb-contaminated soils may be of environmental concern because of the increased risk of groundwater contamination via metal leaching (Meers et al., 2005). For example, Grc−man et al. (2003) reported that 38% of the initial Pb was leached out of the soil column soon after treatment with 10 mmol kg⁻¹ EDTA.

Summary

From the obtained results, our data could be summarized as in the presence of chelators, heavy metals accumulation in shoot and root depending on the type and concentration of chelator(s) applied. Also, it was noticed that DTPA was the most effective chelators material in solubilization of Pb and Ni compared to other chelating agents applied. Nevertheless, in some cases, however, citric acid showed priority in Ni phytoextraction. Under our experimental condition, this study provides evidence for minimizing hazards of heavy metals in soil system by using such chelator materials to enhance phytoextraction of mustard plant from contaminated soils.
References


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تأثير إضافة المخلبيات الصناعية والحامض العضوي المنخفض في الوزن الجزئي على تشجيع امتصاص الرصاص والنيكل بواسطة نبات الخردل

أحمد سيد تعلب، علاء محمد زغلول و فريدريك جيرارد قسم تغذية النبات والقسم الأراضي واستغلال المياه المركز القومي للبحوث الجزيرة، مصر.

أنا - مونتيليا - فرنسا.

أقيمت تجربة في الصوبة والهدف من هذه الدراسة هو تقييم تأثير إضافة مخلبيات حمض الستريك على امتصاص الرصاص والنيكل من التربة وتشجيع امتصاصهما بواسطة نبات الخردل.

باشرت النتائج المتحصل عليها أن إضافة هذه الأنواع المختلفة من المخلبيات أدت إلى انخفاض معنوي في وزن المادة الجافة للنباتات بالمقارنة بال kontrol. وأظهرت أيضاً أن العوامل التي تحكم في امتصاص النبات للعناصر الثقيلة هي نوع المادة المخلبية ومعدل إضافتها حيث ادى إضافة المعدل العالي من DTPA، EDTA إلى زيادة معنوية في تركيز الرصاص في المجموع الخضري. نفس الاتجاه كان ملاحظ مع عنصر النتيل. ووضحت النتائج أن تركيز الرصاص والنيكل داخل النبات كان مرتبطاً مع الكمييات المستخلصة من التربة.

نستخلص من ذلك أنه يمكن تشجيع استخلاص العناصر الثقيلة من التربة الملائمة بواسطة نبات الخردل عن طريق استخدام المخلبيات الصناعية التي تساعد على زيادة امتصاص العناصر الثقيلة من التربة.