Chemically enhanced phytoextraction of Pb by wheat in texturally different soils

Saifullah a,b, Munir Hussain Zia c,d, Erik Meers e,*, Abdul Ghafoor a, Ghulam Murtaza a, Muhammad Sabir a, Muhammad Zia-ur-Rehman a, F.M.G. Tack e

a Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan
b Soil and Water Science Department, Institute of Food Science and Agriculture, University of Florida, Gainesville, USA
c Technical Services Department, Fauji Fertilizer Company Limited, 11 Shahrah-e-Aiwan-e-Tijarat, Lahore, Pakistan
d USDA-Agricultural Research Service, 10300 Baltimore Avenue, Beltsville, MD 20705, USA
e Laboratory of Analytical Chemistry and Applied Ecochemistry, Ghent University, Belgium

A pot study was used to examine the effects of amendments such as EDTA and elemental sulfur on the growth potential, gas exchange features, uptake and mobilization of Pb by wheat (Triticum aestivum L.) in two texturally different contaminated soils at three levels of EDTA (2, 4, 8 mmol kg−1 dry soil) and two levels of elemental sulfur (100, 200 mmol kg−1 dry soil). EDTA resulted in more solubilization of Pb than elemental sulfur in both soils. Application of EDTA and elemental sulfur increased shoot dry matter in the loamy sand soil, whereas in the sandy clay loam soil EDTA treated plants produced lower shoot dry matter compared to that observed with elemental sulfur. Application of EDTA 10 d prior to harvest increased the amount of Pb accumulated into wheat shoots with more Pb accumulated by plants from the loamy sand than from the sandy clay loam soil. However, evaluation of the relative extraction efficiency expressed as the percentage of solubilized Pb that is subsequently also effectively accumulated by the plant shoots reveals that the relatively low efficiency does not warrant the massive mobilization induced by the environmentally persistent EDTA chelator. More modest mobilization of Pb induced by elemental sulfur and the higher relative extraction of mobilized Pb therefore deserves further attention in future research. In particular, attention needs to be paid to determining soil types in which elemental sulfur can induce significant impact on soil pH and metal mobility after application of a practically realistic dosage.

© 2010 Published by Elsevier Ltd.

1. Introduction

Phytoremediation, especially phytoextraction has emerged as a cost-effective, environmental benign technology for the restoration of metal contaminated soils (Salt et al., 1998). Phytoextraction is a green technology that uses plants to remove inorganic contaminants, primarily heavy metals, from soils and waters. Phytoextraction can be broadly classified as either natural or chemical-assisted. The natural phytoextraction strategy utilizes metal hyperaccumulating plant species (Kumar et al., 1995). To date, there are over 420 vascular plants that have been identified as hyperaccumulating species (Roosens et al., 2003). The chemically assisted phytoextraction approach makes use of high yielding plant species. These species lack the inherent ability to take up large concentrations of heavy metals as characterized by hyperaccumulating species, however they can potentially accumulate large amounts when grown in soils that have been chemically treated with chelating agents (Meers et al., 2004; Saifullah et al., 2009a).

The success of phytoextraction, either natural or chemically assisted, is largely determined by plant biomass, metal concentration in plant tissue and phytoavailable fraction of metals in rooting medium. Heavy metals in soils are intimately associated with various soil components and their mobility and availability is determined largely by the way metals are bound to these soil components. Specifically, the migration and phytoavailability of heavy metals in soils has been correlated with soil texture (clay content), surface area, cation exchange capacity (CEC) and soil pH (Kabata-Pendias and Pendias, 2001). For example, the mobility and phytoavailability of metals in soils has been correlated with soil texture (clay content), surface area, cation exchange capacity (CEC) and soil pH (Kabata-Pendias and Pendias, 2001). For example, the mobility and phytoavailability of metals in soils has been correlated with soil texture (clay content), surface area, cation exchange capacity (CEC) and soil pH (Kabata-Pendias and Pendias, 2001). For example, the mobility and phytoavailability of metals in soils has been correlated with soil texture (clay content), surface area, cation exchange capacity (CEC) and soil pH (Kabata-Pendias and Pendias, 2001). For example, the mobility and phytoavailability of metals in soils has been correlated with soil texture (clay content), surface area, cation exchange capacity (CEC) and soil pH (Kabata-Pendias and Pendias, 2001). For example, the mobility and phytoavailability of metals in soils has been correlated with soil texture (clay content), surface area, cation exchange capacity (CEC) and soil pH (Kabata-Pendias and Pendias, 2001).
uptake of a target metal and its translocation to shoots. This is particularly true for Pb due to its strong binding affinity with soil constituents and precipitation as carbonates, hydroxides and phosphates (McBride, 1994). Therefore, Pb-contaminated soils are difficult to remediate with natural phytoextraction that utilizes hyperaccumulators because natural hyperaccumulator plant species generally exhibit slow growth and small biomass making the remediation process effective over a long time. Alternatively, high biomass producing plants can be successfully used for remediation if Pb concentrations are increased in the soil solution with the application of certain chemical additives.

In order to enhance the availability of Pb in soil and translocation from root to shoot, application of chelating agents such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), pyridine-2,6-dicarboxylic acid, trans-1,2-diaminocyclohexane-N,N,N′,N′-tetracacetate (CDTA), ethylenediamine disuccinate (EDDS) have been proposed (Huang et al., 1997; Kayser et al., 2000; Puschenreiter et al., 2001; Grömann et al., 2003; Meers et al., 2005b; Saifullah et al., 2009b). These agents influence the distribution of metals in soils by converting them from insoluble to soluble fractions (Liu et al., 2006). EDTA has the potential to release of metals from insoluble solid phases by forming dissolved complexes due to its very high binding affinity with metals when applied at higher concentrations (Nowack, 2002). Similarly application of elemental sulfur could result in the solubilization of insoluble metals, thus increasing the soil solution concentration, by lowering soil pH following microbiologically driven oxidation of elemental sulfur into sulfate (Kayser et al., 2000). Subsequently, metals in the soil solution could be readily absorbed by plant roots and translocated to the shoots. Although a number of studies have been conducted on the chemically enhanced phytoextraction of metals, there is paucity of information regarding behavior of amendments in texturally different soils. In order to understand the effects of soil texture on the performance of EDTA and S, the present study was initiated with two specific objectives, (1) to assess the effects of different chemical amendments (EDTA, S) on the growth and gas exchange of wheat grown in two texturally different Pb-contaminated soils and (2) to assess the comparative role of EDTA and S for enhancing solubility and uptake of Pb by wheat in two Pb-contaminated soils of differing in texture.

2. Materials and methods

2.1. Study area and sampling

Surface soil samples (0–20 cm) were collected from an agricultural field irrigated with untreated city sewage located at a village 217-RB, Kajlianwala, Faisalabad–Pakistan. After air-drying, the composite soil samples were sieved <2 mm. Soil samples were analyzed for saturation paste pH (pH5), pH of soil to water ratio (pH1:2), saturation paste extract EC (ECs), organic matter (OM), lime content (CaCO3), soil texture, and cation exchange capacity (CEC) following methods described by the US Salinity Laboratory Staff (1954) and Page et al. (1982). The total soil Pb concentrations were determined by digesting soil samples in a mixture of HCl/HNO3 (McGrath and Cunliffe, 1985). Water soluble metal content in soil was measured after extraction of dry soil (10 g) with deionized (DI) water (50 mL) on an orbital shaker at 200 rpm for 16 h. After centrifugation (10000 rpm for 15 min), water extracts were filtered through Whatman No. 42 filter papers before analysis by flame atomic absorption spectrometry (FAAS) (Santos et al., 2006). The reliability of the digestion and analytical procedure was tested via the inclusion of blanks with every batch of sample digest. Reagent blanks and at least two replicates of all samples were used to ensure accuracy and precision of the analysis.

The first soil used in the study (Table 1) was loamy sand in texture, alkaline (pH5 = 7.95) and calcareous (CaCO3 = 9.2 g kg–1) with ECs of 2.05 dS m–1 and sodium adsorption ratio (SAR) of 1.60. The ECs and SAR suggest that the soil was normal according to the classification of the US Salinity Laboratory Staff (1954) for salt-affected soils. The second soil used in the study was sandy clay loam in texture, alkaline (pH5 = 8.2) and calcareous (CaCO3 = 17.8 g kg–1) with an ECs of 3.5 dS m–1 and SAR of 16.0. The ECs and SAR suggest that this soil was slightly sodic according to the classification of the US Salinity Laboratory Staff (1954) for salt-affected soils.

2.2. Preparation of soil for wheat greenhouse study

Soil was spread over a polyethylene sheet and a Pb(NO3)2 salt solution was sprayed over a thin layer of soil to obtain a Pb concentration of 500 mg kg–1 soil. The soil was subsequently thoroughly mixed to achieve uniformity in Pb spiking and was stored in large containers lined with a polyethylene sheet prior to use. The soil was maintained at moisture content near saturation and allowed to equilibrate with periodic mixing for 3 weeks. Following this incubation period, the soil was air-dried and soil samples were taken to determine the extractability of Pb from the spiked soils (Table 1).

2.3. Pot experiment – wheat greenhouse experiment

Seeds of the wheat variety Auqab-2000 were obtained from the Ayub Agricultural Research Institute, Faisalabad. The loamy sand and sandy clay loam soils spiked with Pb (500 mg kg–1) were used for this experiment. Amendments used for enhancing Pb availability and uptake were elemental sulfur and EDTA. Three levels of EDTA (2, 4, 8 mmol kg–1 dry soil) and two levels of elemental sulfur (100, 200 mmol kg–1 dry soil) were applied to each soil. Each treatment was triplicated according to a completely randomized design. Three control pots without amendments but with Pb (500 mg kg–1) were maintained to compare the treatment effects. Before filling the experimental pots with air-dried soil (11.5 kg), elemental sulfur was thoroughly mixed with the soil, which was fertilized with 75 mg N kg–1 (urea), 100 mg P kg–1 (single superphosphate) and 70 mg K Kg–1 (KCl). Ten seeds per pot were initially sown at December 21, 2005, however, finally only five seedlings per pot were maintained up to the bolting stage with the uprooted seedlings being crushed and mixed into the same pot. A second dose of fertilizer nitrogen at 75 mg N Kg–1 was applied 25 d after germination with irrigation water. Ten days before harvesting, EDTA was applied as a solution to the surface of the soils. Two hours before harvesting on April 20, 2006, measurements of

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical and chemical characteristics of soils.</strong></td>
</tr>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Textural class</td>
</tr>
<tr>
<td>Sand (%)</td>
</tr>
<tr>
<td>Silt (%)</td>
</tr>
<tr>
<td>Clay (%)</td>
</tr>
<tr>
<td>pH5</td>
</tr>
<tr>
<td>pH1:2</td>
</tr>
<tr>
<td>ECs (dS m–1)</td>
</tr>
<tr>
<td>SAR (mmol L–1)1/2</td>
</tr>
<tr>
<td>CEC (cmol, kg–1)</td>
</tr>
<tr>
<td>CaCO3 (g kg–1)</td>
</tr>
<tr>
<td>OM (g kg–1)</td>
</tr>
<tr>
<td>Pb (mg kg–1) (before incubation)</td>
</tr>
<tr>
<td>Pb (mg kg–1) (after incubation)</td>
</tr>
</tbody>
</table>

pH5 by saturated soil paste extract, pH1:2 by soil to water ratio (1:2).

* Total metals, while values in parenthesis represent water soluble metals.
2.4. Determination of gas exchange features

Measurements of photosynthetic and transpiration rate were performed at mid-morning on fully expanded flag leaves of three randomly selected plants from each pot. Measurements were made using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The LCA-4 analyzer was used in conjunction with portable leaf chambers to measure various parameters associated with the process of photosynthesis. Such measurements were conducted in an open system configuration in which fresh air was continuously passed through the chamber. The experimental conditions for gas exchange features were set as follows: leaf surface area 11.35 cm², ambient CO₂ concentration (C₀) = 406 µmol mol⁻¹, temperature of leaf chamber ranged from 31.8 °C to 38.5 °C, chamber gas flow rate = 408 mL min⁻¹, molar flow of air per unit leaf area = 251 - µmol s⁻¹, ambient pressure = 992 kPa, water vapor pressure in the chamber ranged between 21 and 24 kPa, and photosynthetically active radiation (Q leaf) at leaf surface was maximum up to 1122 E.

2.5. Plant tissue and soil analysis

Plants were harvested 1 cm above the soil surface and were washed sequentially with 1% HCl, tap water and distilled water to remove any adhering material. After blotting with tissue, fresh weight of shoots was recorded and thereafter plant material was air-dried for 2 d under shade. The air-dried plants were then oven dried at 65 ± 5 °C until there was no change in dry mass. Dried plant material was ground to a fine powder in a mechanical grinder (MF 10 IKA, Werke Germany) to pass through a 1 mm sieve. A portion of plant sample (0.5 g) was digested in a diacid mixture of nitric acid and perchloric acid (3:1) on a hot plate (Miller, 1998). Concentrations of Pb, in the digested solutions was determined using FAAS (Model Thermo S-Series). Soil samples were taken from pots immediately after plant harvest to determine the DI extractable Pb. Water soluble metal content in soil was measured after extraction of 10 g of dry soil with 50 mL DI water in an orbital shaker at 200 rpm during 16 h. After centrifugation, water extracts were filtered through Whatman No. 42 for onward analysis by FAAS (Santos et al., 2006).

2.6. Statistical analysis

All statistical analyses were performed using MINITAB version 14 software. Experimental results were compared using a general linear model. The data gathered were analyzed statistically following ANOVA. Beyond ANOVA least square means and then standard errors were reported. The differences were statistically significant when p < 0.05.

3. Results

3.1. Effect of amendments on crop biomass and gas exchange features

There were significant (p < 0.01) main and interactive effects of amendments and soils as well as treatment × soil interaction on shoot dry matter (SDM) (Table 2). The SDM in response to amendments was the lowest with control treatment and the highest with elemental sulfur at 200 mmol kg⁻¹ for loamy sand soil. The SDM yield at harvest was significantly (p < 0.01) higher from the loamy sand than from the sandy clay loam soil. Moreover, in loamy sand soil, elemental sulfur performed better in enhancing crop biomass but in sandy clay loam soil none of the treatments significantly increased crop biomass compared to control treatment.

There were significant main and interactive effects of amendments (p < 0.01), soils (p < 0.01) and amendment × soils interaction (p < 0.01) on plant photosynthesis rate (Table 2). The photosynthetic rate in response to amendment application ranged between 17.3 µmol m⁻² s⁻¹ for the control (500 mg Pb kg⁻¹ dry soil) to 22.2 µmol m⁻² s⁻¹ for elemental sulfur at 200 mmol kg⁻¹ for loamy sand soil. For sandy clay loam soil, the plant photosynthesis rate ranged from 16.8 µmol m⁻² s⁻¹ for EDTA at 8 mmol kg⁻¹ to 18.5 µmol m⁻² s⁻¹ for the control treatment. The photosynthetic rate increased with increasing application rate of EDTA and elemental sulfur in loamy sand soil. However, in sandy clay soil while the photosynthetic rate increased with the application rate of elemental sulfur it decreased with an increasing application rate of EDTA.

There were significant main and interaction effects of amendments (p < 0.01), soils (p < 0.01) and amendment × soils interaction (p < 0.01) on plant transpiration rate (Table 2). The transpiration rate in response to amendment application ranged between 1.67 mmol H₂O m⁻² s⁻¹ for the control (500 mg Pb kg⁻¹ soil) and 2.79 mmol H₂O m⁻² s⁻¹ for elemental sulfur at 200 mmol kg⁻¹ for the loamy sand soil. For the sandy clay loam soil, the transpiration rate ranged from 1.55 mmol H₂O m⁻² s⁻¹ for elemental sulfur at 100 mmol kg⁻¹ to 2.02 mmol H₂O m⁻² s⁻¹ for the control treatment.

3.2. Effect of amendments on Pb extraction by deionized water and soil pH

The concentration of Pb extracted by deionized water (Table 3) showed significant (p < 0.01) effects of amendments, soils and interaction (amendment × soils). In general, EDTA was the best

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot dry matter (g pot⁻¹)</th>
<th>Photosynthetic rate (µmol m⁻² s⁻¹)</th>
<th>Transpiration rate (mmol H₂O m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loamy sand</td>
<td>Sandy clay loam</td>
<td>Loamy sand</td>
</tr>
<tr>
<td>Control</td>
<td>27.8 ± 0.4</td>
<td>31.8 ± 0.44</td>
<td>17.3 ± 0.70</td>
</tr>
<tr>
<td>E-2</td>
<td>29.5 ± 0.31</td>
<td>31.0 ± 0.10</td>
<td>17.5 ± 0.86</td>
</tr>
<tr>
<td>E-4</td>
<td>29.6 ± 0.26</td>
<td>30.1 ± 0.25</td>
<td>18.3 ± 0.31</td>
</tr>
<tr>
<td>E-8</td>
<td>31.7 ± 0.70</td>
<td>9.8 ± 0.47</td>
<td>18.6 ± 0.52</td>
</tr>
<tr>
<td>S-100</td>
<td>5.0 ± 0.27</td>
<td>30.7 ± 0.10</td>
<td>20.5 ± 0.36</td>
</tr>
<tr>
<td>S-200</td>
<td>39.3 ± 0.24</td>
<td>30.9 ± 0.05</td>
<td>22.28 ± 0.18</td>
</tr>
</tbody>
</table>

ANOVA: Treatment p < 0.01, Soil p < 0.01, Treatment × Soil p < 0.01.

E = EDTA, S = elemental sulfur and numbers following E and S are application rates of amendments in mmol kg⁻¹. The probability level of significant difference is at p = 0.05. Values shown are mean ± SE, where n = 3.
amendment for enhancing Pb extraction by DI water. Significantly more Pb was found in the soil solution of the loamy sand compared to that of the sandy clay loam soil. The DI water extractable Pb in response to amendments ranged from 0.98 mg kg\(^{-1}\) with elemental sulfur at 100–290 mg kg\(^{-1}\) with EDTA at 8 mmol kg\(^{-1}\) for the loamy sand soil. For the sandy clay loam soil, DI water extractable Pb ranged from 0.38 mg kg\(^{-1}\) with elemental sulfur at 100 mmol kg\(^{-1}\) to 238 mg kg\(^{-1}\) with EDTA at 8 mmol kg\(^{-1}\). DI water extractable Pb, decreased in the order E-8 > E-4 > E-2 > S-200 > control > S-100 for both soils indicating that application of S at lower rate was ineffective in enhancing solubility of Pb when compared with any tested level of EDTA.

The soil pH at wheat harvest (Table 3) was significantly (\(p < 0.01\)) related to amendments, soils and amendments × soils. The elemental sulfur proved better than EDTA at decreasing pH in both soils. Even more so, EDTA had an increasing effect on soil pH in the sandy clay loam soil. The decrease in pH with amendments was more pronounced for the loamy sand than for the sandy clay loam soil. This was attributed to a lower calcium carbonate contents in the coarse textured soil.

### 3.3. Effect of amendments on phytoextraction of Pb

For shoot Pb concentration, there were significant (\(p < 0.01\)) main and interactive effects of amendments, soils and amendments × soils (Table 4). For both soils (loamy sand and sandy clay loam), the highest Pb concentration was observed in plant shoots treated with EDTA at 8 mmol kg\(^{-1}\) soil, while, the lowest concentration was observed in plants where elemental sulfur was applied at 100 mmol kg\(^{-1}\). The results indicated that elemental sulfur was an ineffective amendment for increasing shoot Pb concentration when compared to that of EDTA for both soils. The effect of EDTA and elemental sulfur on shoot Pb concentration was more pronounced for loamy sand rather than for sandy clay loam. The Pb uptake by wheat shoots (Table 4) was significantly (\(p < 0.01\)) related to main and interactive effects of amendments, soils and amendments × soils. For both soils, i.e., sandy clay loam and loamy sand, elemental sulfur was ineffective at increasing Pb uptake by wheat plants.

### 4. Discussion

#### 4.1. Effect of amendments on Pb mobility and soil pH after wheat harvest

The concentration of DI water extractable Pb for the untreated controls was 2.99 and 0.99 mg kg\(^{-1}\) in the loamy sand and the sandy clay loam, respectively. For both soils, in agreement with reports by Cui et al. (2004a) and Wang et al. (2007), EDTA was much more effective in Pb solubilization than elemental sulfur. Cui et al. (2004a) reported only a 2.7-fold increase in soluble Pb following the application of elemental sulfur at 160 mmol kg\(^{-1}\) soil relative to a control treatment. In comparison, application of EDTA at 8 mmol kg\(^{-1}\) resulted in 342-fold increase in soil solution Pb relative to the control treatment. The Pb concentration in soil solution increased with the application rate of EDTA for both soils. Several authors have reported a positive relationship between EDTA application rate and metal removal from soils (Papassiopi et al., 1999; Wenzel et al., 2003). For instance, Kim et al. (2003) studied the effects of solution to soil ratio, major cations present in soils and the

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water soluble Pb concentration (µg g(^{-1}))</th>
<th>Soil pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loamy sand</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Control</td>
<td>1.99 ± 0.02</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>E-2</td>
<td>92.1 ± 2.35</td>
<td>92.7 ± 1.33</td>
</tr>
<tr>
<td>E-4</td>
<td>133 ± 0.74</td>
<td>128 ± 2.17</td>
</tr>
<tr>
<td>E-8</td>
<td>209 ± 2.79</td>
<td>238 ± 2.72</td>
</tr>
<tr>
<td>S-100</td>
<td>0.98 ± 0.04</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>S-200</td>
<td>8.39 ± 0.04</td>
<td>5.13 ± 0.46</td>
</tr>
</tbody>
</table>

**ANOVA**

- Treatment: \(p < 0.01\)
- Soil: \(p < 0.01\)
- Treatment × soil: \(p < 0.01\)

**E = EDTA, S = elemental sulfur and numbers following E and S are application rates of amendments in mmol kg\(^{-1}\). The probability level of significant difference is at \(p = 0.05\). Values shown are mean ± SE, where \(n = 3\). The pH values are range of three values.**

### Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pb concentration (µg g(^{-1}))</th>
<th>Pb uptake (mg pot(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loamy sand</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Control</td>
<td>10.5 ± 0.14</td>
<td>16.5 ± 0.57</td>
</tr>
<tr>
<td>E-2</td>
<td>54.1 ± 0.56</td>
<td>53.3 ± 0.45</td>
</tr>
<tr>
<td>E-4</td>
<td>56.4 ± 0.80</td>
<td>58.0 ± 0.35</td>
</tr>
<tr>
<td>E-8</td>
<td>91.0 ± 0.70</td>
<td>70.8 ± 1.03</td>
</tr>
<tr>
<td>S-100</td>
<td>96.6 ± 0.27</td>
<td>4.28 ± 0.14</td>
</tr>
<tr>
<td>S-200</td>
<td>12.8 ± 0.42</td>
<td>8.41 ± 0.10</td>
</tr>
</tbody>
</table>

**ANOVA**

- Treatment: \(p < 0.01\)
- Soil: \(p < 0.01\)
- Treatment × soil: \(p < 0.01\)

**E = EDTA, S = elemental sulfur and numbers following E and S are application rates of amendments in mmol kg\(^{-1}\). The probability level of significant difference is at \(p = 0.05\). Values shown are mean ± SE, where \(n = 3\).**
 EDTA to Pb stoichiometric ratio on the extraction of Pb using different Superfund soils and reported that extraction of Pb from Pb-contaminated soils was not affected by the water:soil ratio (as low as 3:1) but was dependent on the quantity of EDTA applied. Application of EDTA in a sufficiently large amount (EDTA:Pb stoichiometric ratio >10) resulted in extraction of most of the Pb from soil.

In the current study higher extraction efficiencies were observed for loamy sand than for sandy clay loam soil. The addition of EDTA at 8 mmol kg\(^{-1}\) to the soils led to a 97-fold (loamy sand) and a 240-fold (sandy clay loam) increase in soluble Pb relative to the control treatment. However, of the total soil Pb applied (500 mg kg\(^{-1}\)), EDTA resulted in more solubilization of Pb in the loamy sand than that in the sandy clay loam soil. For the loamy sand soil, application of EDTA at 8 mmol kg\(^{-1}\) solubilized 58% of the total applied Pb, whereas for the sandy clay loam, the same rate of EDTA solubilized only 47% of the total applied Pb. The lower effectiveness of EDTA extraction in the sandy clay loam soil may be attributed to a higher silt/clay fraction compared to that of the loamy sand soil. Hoola and Alloway (1994) reported that fine textured soils generally exhibited more metal retention than coarse textured soils. In general, all other factors being similar, soils with a high clay contents tend to retain higher amounts of trace elements than coarse textured soils, since clays have the unique feature suitable for trapping pollutants (metal ions) from the environment including high specific surface area associated with their small size and competition to produce higher degrees in all soils. Clays can adsorb heavy metals via ion exchange reactions and by the formation of inner sphere complexes at the surface of clay particles (Peters and Shem, 1992). Peters and Shem (1992) studied the adsorption/desorption of Pb onto various types of soils both in the presence and absence of EDTA and noted that soil with the highest silt/clay contents exhibited the greatest Pb adsorption.

Application of acids or acidifying materials could significantly increase metal solubility and availability via lowering of soil pH (Huang et al., 1997). In this study application of elemental sulfur decreased pH in both the soils. The magnitude of this decrease increased with sulfur dosage. Similar results, a decrease in soil pH with application of elemental sulfur, have previously been reported (Kaysen et al., 2000; Cui et al., 2004a,b; Velarde et al., 2005; Wang et al., 2007). The effectiveness of sulfur application in lowering soil pH and subsequently increasing metal availability seems to be principally due to the microbially driven oxidation of elemental sulfur to sulfuric acid. This process, metal mobility is increased as protons bind to oxide ions on the surface of soil particles. As this oxidation process progresses, other bonds responsible for binding metal ions are weakened and this allows easier detachment of metal species into soil solution (Sparks, 1995). In the current study, decrease in pH was more pronounced in loamy sand than rather than in sandy clay loam. This may be attributed to a higher clay percentage as well as buffering capacity of the sandy clay loam soil compared to that of the loamy sand (Table 1). These results are in line with the findings of Neilsen et al. (1993) who reported that the magnitude of pH decrease in response to added sulfur, varied inversely with initial CaCO\(_3\) contents. Similarly McCready and Krouse (1982) noted that the pH of surface soil changed little as a result of the S oxidation due to the buffering capacity and presence of lime in tested soils.

4.2. Effect of amendments on crop biomass and gas exchange features

The efficiency of phytoextraction depends both upon the metal concentration in shoots and biomass production (McGrath et al., 2002; Lesage et al., 2005). In the current study, application of EDTA and S increased plant biomass from the loamy sand compared to that from the sandy clay loam. However, S was much more effective than EDTA for enhancing plant biomass production. However, increase in shoot biomass with the application of S was more pronounced in only loamy sand compared to EDTA. EDTA is not essential for plant growth but could affect plant biomass directly or indirectly by affecting the phytoavailability of heavy metals. Elemental sulfur has a variety of uses as a soil amendment, and its oxidation to H\(_2\)SO\(_4\) is particularly beneficial in alkaline soils to decrease pH, supplying SO\(_4\) to plants, and making P and micronutrients more available in soils.

In our study, higher dry matter production with the application of elemental sulfur may be attributed to a decrease in soil pH which might have resulted in better availability of nutrients to plants. Velarde et al. (2005) also found that elemental sulfur was highly effective for increasing shoot biomass. However, findings of this study are contrary to those reported by Hammer et al. (2003), Cui et al. (2004a, b). Cui et al. (2004a) reported 50% and 60% reduction in biomass of Indian mustard and winter wheat compared to control, respectively with the application of S at 160 mmol kg\(^{-1}\). The reason for the discrepancy between these studies may be due to more pronounced effect of S on solubility and availability of heavy metals in the study of Cui et al. (2004a) when compared to the present study. For the sandy clay loam soil in the present study, S amendment was not as effective as in the loamy sand soil. This may be attributed to a higher initial pH as well as higher buffering capacity of the sandy clay loam compared to that of the loamy sand soil which resulted in lower availability of macro- and micronutrients like P and Mn. Interestingly application of EDTA did not significantly affect plant biomass production even at its highest rate of application. This is contrary to findings of other investigators (Ruley et al., 2006; Hernandez-Allica et al., 2007) who reported decreased yields of cardoon plants with the application of EDTA at higher rates. The observed metal mobility in response to EDTA application (Table 3), indicates that EDTA is better than S for the solubilization of Pb and despite mobilizing higher concentrations of Pb, EDTA did not affect the plant biomass. Therefore, it seems that EDTA in soil might have complicated most of the Pb rendering it non toxic. This may apply particularly for the current experimental condition, in which sandy soil types are spiked with Pb(NO\(_3\))\(_2\) which under non-aged conditions would lead to an increased relative free ion activity of Pb\(^{2+}\) in the soil solution. Chelation to EDTA could subsequently have resulted in a less toxic Pb speciation.

With respect to gas exchange features both EDTA and S amendments produced contrasting effects. Moreover, the soil type had a significant effect on the observed gas exchange features. Application of EDTA and S significantly increased the photosynthesis and transpiration rates compared with that of the control treatment in the case of the loamy sand soil. However, in the sandy clay loam soil, both these amendments resulted in significantly lower photosynthesis and transpiration rates. Plant roots can more easily extract water and nutrients from coarse textured soils when compared to fine textured soils resulting in better plant growth. In the current study, deterioration in soil structure was observed visually due to the dispersion of clay particles following the application of EDTA to the sandy clay loam soil. This dispersion might have resulted in less plant growth as was evident by the low photosynthesis and transpiration rates in sandy clay loam soil.

4.3. Phytoextraction of Pb

In the present study, soil applied EDTA at all rates significantly increased Pb accumulation in wheat shoots relative to the control treatment. The effectiveness of EDTA in enhancing Pb concentration and total Pb uptake in wheat shoots was considerably higher from the loamy sand than from the sandy clay loam soil. Addition of EDTA to loamy sand soil at 2, 4 and 8 mmol kg\(^{-1}\) increased the
concentration of Pb in shoots by 5.1–5.4- and 7.7-fold, respectively over the control plants. While in the sandy clay loam soil, the corresponding increase in shoot Pb concentration was 3.2–3.5- and 4.3-fold over the control plants. The increase in Pb uptake with EDTA can be explained by its effect on enhancing the solubility of Pb and absorption of the Pb-EDTA complex by plants (Santos et al., 2006; Wang et al., 2007). The increase in Pb uptake with the addition of EDTA was not as high as stated by other investigators (Huang et al., 1997; Schmidt, 2003). Huang et al. (1997) reported over a 100-fold increase in Pb concentration in plant shoots following the application of EDTA. Similarly Blaylock et al. (1997) reported that the concentration of Pb in shoots of Indian mustard increased from <100 to 15 000 mg kg⁻¹ of Pb, when the plants were grown in soil containing 600 mg kg⁻¹ of Pb amended with 10 mmol kg⁻¹ EDTA. The lower effectiveness in Pb uptake may be due to differences in soil types, amendment dose and application timing and difference in plant species used in the experiments. Large differences between reports can be associated to differences in experimental design. For example, Lesage et al. (2005) found detrimental effects of 3 mmol kg⁻¹ EDTA application on biomass productivity of Helianthus annuus when EDTA was applied before sow, while other authors observed an increase in biomass productivity for the species and cultivar used when the same amounts of EDTA were applied just prior to harvest (Meers et al., 2005a).

Lower effectiveness of the EDTA amendment in the sandy clay loam soil relative to the loamy sand soil for enhancing Pb accumulation in wheat shoots may be attributed to the differences in soil properties such as soil texture, lime contents and pH. Plant accumulation of metals depends on the concentration of metals in the soil solution that is directly accessible by the plant roots, and such pools of metals can successfully be increased by the application of chelating agents such as EDTA, CDTA, HEDTA, EDDS, NTA, and DTPA (Ruley et al., 2006; Tandy et al., 2006). In the sandy clay loam soil, EDTA resulted in significantly lower Pb solubilization compared to that observed in the loamy sand soil due to this soils higher clay and lime contents (Table 1). The lower concentration of water soluble Pb in the sandy clay loam soil resulted in a lower shoot Pb concentration as well as uptake by wheat plants compared to that from the loamy sand soil application of S was not as effective as EDTA in enhancing Pb phytoextraction. The lower effectiveness of S compared to that of EDTA could be attributed to its smaller effect on Pb solubility (Table 3).

Based on Tables 3 and 4, the relative fraction of solubilized Pb that is subsequently also effectively phytoextracted can be calculated. In unamended soils, respectively 0.84% and 4.6% of soluble Pb in the loamy sand and sandy clay loam soil is taken up by the plants. When applying EDTA to the soil, soluble Pb increases dramatically, while extraction does not increase in the same order of magnitude. This results in a relative extraction of respectively 0.09–0.15% and 0.07–0.15% of soluble Pb in the loamy sand and sandy clay loam soil. Analogously for the elemental sulfur treatments, relative extraction values are respectively 0.52–3.0% and 0.36–3.0% of soluble Pb in the loamy sand and sandy clay loam soil. This extraction efficiency is considered to be too low to warrant the application of high dosage of Pb mobilizing agents to the soil. Interestingly enough though, the efficiency of sulfur-induced acidification is substantially better than that of the environmentally persistent chelator EDTA. This implies that in the search for more environmentally benign soil amendments to replace EDTA (Meers et al., 2004), elemental sulfur may as yet have a role to play in optimizing soil management to maximize phytoextraction efficiency. In particular, attention needs to be paid to determining soil types in which elemental sulfur can induce significant impact on soil pH and metal mobility after application of a practically realistic dosage.

5. Conclusions

EDTA mobilized Pb more effectively than S in both the soils studied (i.e. loamy sand and sandy clay loam). EDTA resulted in higher Pb mobilization in the loamy sand compared to that of the sandy clay loam soil. Soil pH was decreased with the application of S. The magnitude of the pH decrease was greater in the loamy sand when compared to that observed in the sandy clay loam soil. Application of EDTA and S increased shoot dry matter in the loamy sand soil, while in the sandy clay loam soil, plants treated with EDTA produced lower shoot dry matter compared with S treated plants. Application of EDTA resulted in greater accumulation of Pb when compared to S application. Wheat accumulated more Pb in the loamy sand than in the sandy clay loam soil following the application of EDTA. However, evaluation of the relative extraction efficiency expressed as the percentage of solubilized Pb that is subsequently also effectively accumulated by the plant shoots reveals that the relatively low efficiency does not warrant the massive mobilization induced by the environmentally persistent EDTA chelator. More modest mobilization of Pb induced by elemental sulfur and the higher relative extraction of mobilized Pb therefore deserves further attention in future research. In particular, attention needs to be paid to determining soil types in which elemental sulfur can induce significant impact on soil pH and metal mobility after application of a practically realistic dosage.

References


US Salinity Laboratory Staff, 1954. Diagnosis and Improvement of Saline and Alkali Soils. USDA Handbook 60. Washington, DC, USA.

