Effects of calcium peroxide on arsenic uptake by celery (Apium graveolens L.) grown in arsenic contaminated soil

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ABSTRACT

The ability of calcium peroxide (CaO 2) to immobilize As of contaminated soil was studied using pot and
field experiments. In pot experiment, CaO 2 applied at 2.5 and 5 g kg⁻¹ significantly increased celery shoot
weight and decreased shoot As accumulation, which was ascribed to the formation of stable crystalline Fe
and Al oxides bound As and the reduction of labile As fractions in the soil. The labile As fractions were pH
dependent and it followed a “V” shaped profile with the change of pH. In field experiment, the dose of
CaO 2 application at 750 kg ha⁻¹ was optimal and at which the celery was found to produce the highest
biomass (63.4 Mg ha⁻¹) and lowest As concentration (0.43 mg kg⁻¹). CaO 2 probably has a promising
potential as soil amendment to treat As contaminated soils.

1. Introduction

It is well-known that As is a toxic and carcinogenic element to
human beings, and a number of environmental problems have
been caused by As worldwide (Duker et al., 2005; Williams et al.,
2005; Zhu et al., 2008a, b). This contamination is mostly originated
from mining activity and As leaching produced by the mining
activity, which could be discharged to the surface water or dis-
persed to the surrounding area. Further, the leaching could pene-
trate to the soil lower parts and endanger the groundwater. Soil
is ready to be the recipient of the large amount of As as well. In
China, many soils were contaminated with As, especially those
close to the mining area (Sun, 2004). Extremely high concentra-
tions of As (averaged at 709 and 129 mg kg⁻¹) was reported in agricul-
tural soil surrounding Chenzhou (Hunan Province) and Shantou
(Guangdong Province) mining areas, respectively (Liao et al., 2005;
Liu et al., 2010). Crops and vegetables grown on contaminated soil
can accumulate high levels of As in the roots and shoots (Khan
et al., 2008). Hence, As polluted soil is considered a major source
of contamination in the food chain (Frankenberger, 2002) and the
remediation of As polluted soil is of great importance for reduc-
ing the potential risk of human exposure to As.

Stabilization is regarded as one of the most effective remedia-
tion techniques whereby various amendments are applied to re-
duce As mobility and bioavailability (Moon et al., 2004; Gutierrez
et al., 2010). Lime, iron compounds, type F fly ash, silica fumes,
and blast furnace slag are the common ameliorants used in the
treatment of As contaminated soil (Leist et al., 2000). Among these
amendments, iron compounds were effective in reducing labile As
due to the formation of amorphous iron(III) arsenate (FeAsO₄·H₂O)
(Carlson et al., 2002; Gutierrez et al., 2010). Lime reducing soil As
bioavailability was possibly attributed to the formation of As–Ca
complexes (Moon et al., 2004). As(V) and As(III) are the primary
inorganic arsenic species in environment, but the stabilization of
As(III) is more challenging than As(V). Hence, in the treatment of
As(III)-laden solid waste, it is often necessary to oxidize arsenite
to arsenate prior to stabilization (Fueselle and Taylor, 2004).

Calcium peroxide (CaO 2) is one of the most versatile and safest
substances to handle of the family of solid inorganic peroxy com-
 pounds which are considered a “solid form” of hydrogen peroxy
(Tieckelmann and Steele, 1991). CaO 2 dissolves in H₂O to form
H₂O₂ and Ca(OH)₂ via reaction (1), liberating a maximum of 0.47 g H₂O₂ g⁻¹ CaO 2 (Northup and Cassidy, 2008). The As(III) sta-
bilization process will be improved by CaO 2 application into soil,
because of the reaction of As(III) with H₂O₂ via reaction (2) (Fueselle
and Taylor, 2004). In addition, hydrated lime Ca(OH)₂, the bypro-
ducts of slurring CaO 2, could reduce As mobility due to the formation
of low solubility Ca–As precipitates such as Caₐ(AsO₄)₂·2H₂O and
Caₐ(AsO₄)₃·(OH) (Bothe and Brown, 1999). Therefore, CaO 2
would be a potential amendment for As polluted soil remediation.

CaO₂ + 2H₂O → H₂O₂ + Ca(OH)₂

(CaO₂)
AsO$_4^{3-}$ + H$_2$O + 2OH$^- \rightarrow$ AsO$_3^{2-}$ + 2H$_2$O  

(2)

CaO$_2$ was often used as an alternative oxidant to promote organic pollutant degradation in contaminated soil such as polycyclic aromatic hydrocarbons, 2,4,6-trinitrotoluene, benzene, toluene, ethylbenzene, xylene and so on (Bianchi-Mosquera et al., 1994; Arienzo, 2000; Bogan et al., 2003; Hanh et al., 2005). However, few studies were carried out with CaO$_2$ to stabilize As in the soil.

In the present research, a field around Lianhuashan tungsten mine was selected for studying the ability of CaO$_2$ for arsenic amendment. Lianhuashan tungsten mining area was seriously polluted by As due to the flooding of acid mining water. Our previous studies showed that the As concentrations in agricultural soils were ranged from 35.5–935 mg kg$^{-1}$ with the mean value of 129 mg kg$^{-1}$ and the As concentration reached up to 2.38 mg kg$^{-1}$ for vegetable samples (Liu et al., 2010). Celery (Apium graveolens L.) is a common vegetable locally and it is can accumulate high As concentration in shoots (Huang et al., 2006). The objectives of the present study are to evaluate the potential of CaO$_2$ as soil amendments to immobilize As in the soil and reduce the uptake of As by celery based on pot and field studies. Insights on the As stabilization mechanism and useful information of effective way to deal with the contamination of As can be better understood.

2. Materials and methods

2.1. Pot experiments

Soil used in pot experiments was collected from a paddy field contaminated with arsenic vicinal Lianhuashan tungsten mine (soil were taken from field experiment site). The selected physical and chemical properties are presented in Table 1. Air dried soils were passed through a 2 mm diameter sieve. Basal fertilizers applied to the soils were 100 mg N kg$^{-1}$ dry soil as urea, 80 mg P kg$^{-1}$ and 100 mg K kg$^{-1}$ as KH$_2$PO$_4$ (Shen et al., 2002). CaO$_2$ was added to the soils at the concentrations of 0, 0.5, 2.5, 5, 10 g kg$^{-1}$. After undergoing three cycles (10 d per cycle) of saturation with deionized water and air-drying, soils with different CaO$_2$ amounts were placed in plastic pots at 1 kg soil per pot (height: 25 cm, diameter: 8.5 cm) and each treatment was replicated three times. Before seeding, 10 g soils were sampled from each pot for As fraction and pH analysis.

Seeds of celery were purchased from the Vegetable Research Institute of Guangdong Agricultural Academy. After surface-sterilized in 0.5% NaOCl and rinsed thoroughly with deionized water, seeds were directly sowed in the pots. About 7 d later, 5 plants with uniform size was kept in each pot. The plants were placed in the greenhouse at a totally random arrangement and plants were watered daily to 65% of the field water capacity. The experiments were carried out in a glasshouse at 20–25°C. After 50 d growth, the shoots were cut, washed in tap water thoroughly and then with deionized water. The samples of plants were dried at 80°C for 72 h, and the weight was measured, the content of As was analyzed.

2.2. Field experiments

The field near Lianhuashan tungsten mine contaminated with As was selected for the field experiments. The selected physical and chemical properties are presented in Table 1. The treatment included CaO$_2$ application at the dose of 375, 750 and 1500 kg ha$^{-1}$, and the soil with no CaO$_2$ application was set as the control. Four treatments in a completely-randomized design with four replicates per treatment were set up in experimental plots of 20 m$^2$ (4 × 5 m) each, with 0.5 m distance between plots. The experiments were conducted from November 2009 to January 2010 and in this period the average temperature was 17.2°C (range from approximately 12.3 to 31.4°C).

Celery seedlings of uniform size were selected and about 500 seedlings were planted in each plot. Periodic irrigation with water was applied during the growing season and agricultural measurements such as insecticide and weed were applied when necessary. The plant shoots were harvested on maturity (60 d after growing), and the biomass and As concentration were measured.

2.3. Chemical analysis

2.3.1. Soil property

The soil pH was measured by double-distilled water at a 1:5 ratio (w/v) using a pH meter and the electrical conductivity of the soil was measured using a conductivity meter on the soil extract, obtained by shaking soil with double-distilled water at a water-to-soil ration of 1:5 (w/v) (Thomas, 1996). The soil texture, organic matter content, total N and field water capacity were measured by the procedures described by Avery and Bascomb (1982).

2.3.2. As fractionation

A sequential As fractionation scheme based on the method of Wenzel et al. (2001) was employed to determine the following operationally-defined As fractions: (F1) extractable with 0.05 M (NH$_4$)$_2$SO$_4$ (non-specification sorbed phase), (F2) extractable with 0.05 M NH$_4$H$_2$PO$_4$ (specifically sorbed phase), (F3) 0.2 M NH$_4$+-oxalate buffer-extractable (amorphous Fe and Al oxides bound phase), (F4) 0.2 M NH$_4$+-oxalate buffer + ascorbic acid-extractable crystalline Fe and Al oxides bound phase, and (F5) extractable with HNO$_3$/H$_2$O$_2$ (residual phase).

2.3.3. As analysis

About 0.5 g soil samples were digested by 5 mL of HNO$_3$ and HClO$_4$ at the ratio of 4:1(v/v) with the temperature of 140–160°C and about 1 g plant samples were digested with 1.0 mL of HClO$_4$, 1.5 mL of H$_2$SO$_4$ and 4.0 mL of HNO$_3$ at temperature of 110–130°C until a clear solution was obtained. The digested samples were filtered by 0.45 μm filter, and then analyzed for total arsenic concentrations with a hydrogen generation–atomic fluorescence spectrometer (AFS-820, Beijing Titan Instruments, China). All reagents were of analytical grade or above. To verify the accuracy of metal analysis, certified standards reference materials (GBW/E070008 for soils and GBW10014 for plants) from the National Research Center for Standards in China were used. The certified value of GBW/E070008 and GBW10014 were 75 ± 7 and 0.062 ± 0.014 mg kg$^{-1}$, respectively. Results of this study (n = 12) provide mean values of 72 ± 6 and 0.063 ± 0.017 mg kg$^{-1}$ for GBW/E070008 and GBW10014, which were 97% and 102% of target values, respectively. Reagent blanks and analytical duplicates,

Table 1

<table>
<thead>
<tr>
<th>Physicochemical properties</th>
<th>5.5</th>
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<tbody>
<tr>
<td>pH (H$_2$O)</td>
<td>245</td>
</tr>
<tr>
<td>Electrical conductivity at 25°C (μS cm$^{-1}$)</td>
<td>61.2</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>21.4</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>7.4</td>
</tr>
<tr>
<td>Cation exchange capacity (cmol kg$^{-1}$)</td>
<td>5.48</td>
</tr>
<tr>
<td>Field water capacity (%)</td>
<td>27</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.99</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total As (mg kg$^{-1}$)</td>
<td>162</td>
</tr>
</tbody>
</table>
comprised of 10% of total samples, respectively, were also used where appropriate to ensure accuracy and precision in the analysis.

2.4. Data statistical analysis

Statistical analysis of the experimental data was performed using SPSS 10.0 (SPSS, USA) software. The statistical significance differences were determined by one way analyses of variance on ranks followed by the Student multiple comparison t-test with $p < 0.05$. The correlation analysis was conducted by Pearson correlation, and the level of significance was set as $p < 0.05$ (two-tailed).

3. Results and discussion

3.1. Effects of CaO$_2$ on soil pH and As fractionation in pot experiment

To evaluate the solubility of As in soil treated with CaO$_2$, the pH and As fraction were analyzed. As seen from Table 2, the soil pH significantly increased with the increasing of CaO$_2$ application rates ($p < 0.05$). The highest pH 8.5 was achieved in the treatment of 10 g kg$^{-1}$ CaO$_2$, being about 3 unites higher than that of the control. It was in agreement with the findings of Arienzo (2000) that 0.1–1% (w/w) CaO$_2$ increased the pH of soil slurry from 5.8 to about 12.0. The greatly improved soil pH could be attributed to the hydrated lime generated by CaO$_2$ application (via reaction (1)).

As is a non-essential element for plant and high As concentration in soil can inhibit plant growth and produce visual phototoxic symptoms (Warren et al., 2003; Liu et al., 2004). The toxicity and bioavailability of As depends on plant species and As fractionation in soil as well (Tao et al., 2006). With the same method of As fractionation, Du et al. (2008) found that F1 and F2 in soil were more labile than other fractions. And in vitro experiments, Tang et al. (2007) also found that the As bioaccessibility showed a significantly positive correlation with the sum of As in F1 and F2 fractions, while had a negative correlation with F3, F4 and F5. Accordingly, in this study we differentiated the As fractions of F1 and F2 as labile As (soluble and exchangeable As), and fractions of F3, F4 and F5 as non-labile As (As bound strongly in soil).

Among the five fractions (F1–F5), the highest As concentration was F3 and F5 in each tested soil (Table 2 and Fig. 1). The sum proportions of F3, F4 and F5 as non-labile As (As bound strongly in soil), while had a negative correlation with F3, F4 and F5. The As fractions in soil treated with different dose of CaO$_2$ in pot experiment.

As was more mobile (Magalhães, 2002) due to the As adsorption on soil mineral surface or co-precipitation depending on soil pH (Sadiq, 1997; Al-abed et al., 2007). Fe oxides have the greatest potential for As and heavy metal adsorption in soil (Bowell, 1994; Dixit and Hering, 2003; Luo et al., 2006; Giménez et al., 2009).

10 g kg$^{-1}$ CaO$_2$ added in the soil, the concentration of As in fraction F2 was 22% higher than that of the control. The concentration and proportion of As fraction F3 displayed a reverse trend by the CaO$_2$ application, firstly increased with the increasing CaO$_2$ application from 0 to 2.5 g kg$^{-1}$, then decreased with increasing dosage thereafter (from 2.5 to 10 g kg$^{-1}$ CaO$_2$). The highest concentration and proportion of As fraction F3 were found in the treatment of 2.5 g kg$^{-1}$ CaO$_2$ application which was 1.23 and 1.19 times of the control, respectively. While in 10 g kg$^{-1}$ CaO$_2$ treatment, the concentration and proportion of F3 were significantly lower than that of the control ($p < 0.05$). In the present study, the labile fractions of As decreased significantly with moderate rate of CaO$_2$ application, which might be contribute to that CaO$_2$ application increased the Eh of soil to well-oxidized condition and As(III) would be transform to less mobile specie as As(V) under oxidizing condition (Fuessle and Taylor, 2004; Northup and Cassidy, 2008; Dias et al., 2009). H$_2$O$_2$ liberated from CaO$_2$ application (via reaction (1)) would resolve into H$_2$O and O$_2$ and increase redox potentials with the production of oxygen making arsenite oxidation possible (Fuessle and Taylor, 2004). The As(V) is more effective in forming stable crystalline Fe and Al oxides bound As (As of F3), so that the lability of As reduced by CaO$_2$ treatments through H$_2$O$_2$ releasing. Furthermore, in the presence of Ca under highly oxidizing and moderate pH conditions, calcium hydrogen arsenate (CaHAsO$_4$) and calcium arsenate (Ca$_3$(AsO$_4$)$_2$) could precipitate and reduce the mobility of As (Porter et al., 2004). But under acid or alkaline pH conditions, the As was more mobile (Magalhães, 2002) due to the As adsorption on soil mineral surface or co-precipitation depending on soil pH (Sadiq, 1997; Al-abed et al., 2007). Fe oxides have the greatest potential for As and heavy metal adsorption in soil (Bowell, 1994; Dixit and Hering, 2003; Luo et al., 2006; Giménez et al., 2009).

![Relative distribution of As (%)](image)

Note: Data of pH represent ranges of triplicates and of As fractions represent means of triplicates. Different letters in the same column indicate a significant difference at $p \leq 0.05$ according to the t-test.
Fe oxide surface appears to be positively charged when pH is below its point of zero charge (pzc, pH at which the net surface charge is equal to zero), and vice versa. The pzc for the main Fe oxides in soil, such as goethite, hematite and lepidocrocite, is 7.6–8.1, 6.5–8.6 and 7.8–8, respectively (Bowell, 1994). Under the typical pH conditions (4–9), As(V) is present as the negatively charged oxyanions $\text{H}_2\text{AsO}_4^-$ or $\text{HAsO}_4^{2-}$ (Cheng et al., 2009), and these are attracted to Fe oxides when the mineral surface are positively charged, like when $\text{pH} < \text{pH}_{\text{pzc}}$ (Bowell, 1994). Therefore, the As(V) adsorption would decrease when soil pH increased to higher than $\text{pH}_{\text{pzc}}$. This might be one reason that the labile As were increased when pH increased above about 7 in the present study.

3.3. Celery biomass and As accumulation of celery in pot experiment

The dry weight of celery shoot with different rates of CaO$_2$ application was measured in the pot experiments. As shown in the Fig. 3a, stunted growth of celery was observed in the control (with no CaO$_2$ addition) compared with the CaO$_2$ treatments. The result was consistent with the findings of Gutierrez et al. (2010) that high As concentration ($>20$ mg kg$^{-1}$ of exchangeable As) in soil could strongly inhibit radish growth. The application of CaO$_2$ improved the dry weights of celery shoots and the differences reached significant level when CaO$_2$ application rates were 2.5–5 g kg$^{-1}$. The highest dry weight of celery shoot was 91 g pot$^{-1}$ achieved at CaO$_2$ application rate of 2.5 g kg$^{-1}$ with about 61% increases over the control, which was probably attributed to the lowest labile As concentration (F1 + F2) formation with CaO$_2$ application at this dosage (see Table 2). The As concentration in celery shoots was decreased significantly with the increasing dosage of CaO$_2$ application from 0 to 2.5 g kg$^{-1}$, and then increased with the further increasing dosage of CaO$_2$ application from 2.5 to 10 g kg$^{-1}$ (Fig. 3b). As concentration in celery shoot treated with 0.5, 2.5 and 5 g kg$^{-1}$ CaO$_2$ was significantly lower than that of the control, with 29%, 64% and 23% lower respectively. The As concentration in shoot of celery treated with 10 g kg$^{-1}$ CaO$_2$ was slightly lower than that of control, but the difference was no significant. The minimum As concentration 0.27 mg kg$^{-1}$ was found in the 2.5 g kg$^{-1}$ CaO$_2$ treatments which was about 36% of the control.

Correlation coefficients between As concentration in celery shoot and As five fractions in soil were calculated (Fig. 4). It was found the As concentration in celery shoot was dependent on the As fractionations in soil. The As concentration in celery shoot correlated significantly and positively with F1 ($r = 0.763$, $p < 0.01$), F2 ($r = 0.818$, $p < 0.01$) and the labile As (F1 + F2) ($r = 0.859$, $p < 0.01$), but negatively correlated with F3 ($r = -0.835$, $p < 0.01$) and the sum of the non-labile As (F3 + F4 + F5) ($r = -0.742$, $p < 0.01$). These results were consistent with the results of Gutierrez et al. (2010) who reported that the fact of Ca(OH)$_2$ application significantly decreased labile As fractions of soil might be responsible for the lower As uptake by radish. Du et al. (2008) also found that high As removal by the weak soil extractants (F1 and F2) corresponded to relatively higher bioavailability of As and high concentrations of As in F3 and F4, which represented As sequestered by oxides and resulted in less As bioavailability.

3.4. The implication of CaO$_2$ on As remediation in field experiment

In the field experiment, similar result as that in the pot experiment was observed. CaO$_2$ treatments significantly increased the biomasses of celery shoot, while significantly decreased the As concentration in the shoots of celery (Fig. 5). In comparison with the control, the supply of 375, 750 or 1500 kg ha$^{-1}$ CaO$_2$ led to an increase in fresh weight of celery shoots of 29%, 40% or 17%, respectively; while resulted in a decrease in As concentration of celery shoots of 27%, 61% or 37%, respectively. The optimal dose of CaO$_2$ application was 750 kg ha$^{-1}$, and at this dosage the celery was found with highest biomass (63.4 Mg ha$^{-1}$) and lowest As concentration (0.43 mg kg$^{-1}$).

In the present research, the effects of CaO$_2$ on stabilization of As in contaminated soil were comparable with that of steel-making slag (SMS) reported by Gutierrez et al. (2010) where the application of 2 Mg ha$^{-1}$ SMS decreased As concentration of radish root by 50% in field experiment; and similar to that of zerovalent iron.
reported by Ascher et al. (2009) where the application of 1% zero-valent iron decreased As concentration in lettuce shoots by 59%, from 1.55 to 0.64 mg kg\(^{-1}\) (based on dry weight) in a \textit{in situ} stabilization experiment. However, it was slightly more effective than that of limestone reported by Lee et al. (2011) where the application of 2% limestone decreased As concentration of lettuce shoot by 23%, from 4.36 to 3.38 mg kg\(^{-1}\) (based on dry weight) in pot experiments. The present results found with the optimal dose of CaO\(_2\) application, the As concentration in celery shoots could be decreased to the level obviously lower than that of the local leaf vegetables around the mining area (the As concentration in leaf vegetables ranged from 0.23 to 2.38 mg kg\(^{-1}\) and with the mean of 0.63 mg kg\(^{-1}\) (Liu et al., 2010), and slightly lower than the maximum allowable concentration (MAC) value permitted by the Chinese Government (0.5 mg kg\(^{-1}\), GB15201-94, the Ministry of Public Health of China). It indicated that CaO\(_2\) might be a potential amendment for As stabilization in contaminated soil.

4. Conclusions

The results of pot and field experiments showed that CaO\(_2\) might be a potential amendment for As stabilization in contaminated soil. Moderate CaO\(_2\) application increased celery shoots yield and reduced celery As uptake, which mainly could be attributed to the formation of stable crystalline Fe and Al oxides bound As (As of F3) that decreased the labile and increased non-labile As fractions in soil. The pot experiment results indicated that H\(_2\)O\(_2\) could be liberated from soil and the pH could be changed in soil after CaO\(_2\) was applied to the soil and the formation of crystalline Fe and Al oxides bound As or calcium arsenate might be the reason for reducing the mobility of As under highly oxidizing and moderate pH conditions. But the labile As would increase through As(V) desorption when soil pH increased higher than pH\(_{\text{pzc}}\) with adequate CaO\(_2\) application. Celery grown in soil contaminated with As around Lianhuashan mining area safety for eating according to MAC value permitted by the Chinese Government (GB15201-94) after amended with CaO\(_2\).

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