

Communication

Calcined Oyster Shell-Humic Complex as Soil Amendment to Remediate Cd- and As-Contaminated Soil

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Abstract: Soil acidification in the subtropical region reduces soil productivity and increases the bioavailability of heavy metal(loid)s in soil. Here 800 °C-calcined oyster shell and leonardite-derived humic substance were combined to form a Ca-humic complex to remediate an acidic soil contaminated by cadmium (Cd) and arsenic (As) for safe vegetable production. In the pot experiment, the complex was added to soil as a soil amendment at 0%, 0.5%, 1.0%, 2.0%, and 5.0%. A 2.0% dose reduced Cd and As contents of the vegetable bok choy by 98% and 71%, respectively, alleviating the human health risk of Cd and As from the food chain. The unified bio-accessibility method (UBM) was followed to estimate the bio-accessibility of Cd and As. The complex at a 2.0% dose lowered soil Cd and As bio-accessibility in simulated gastric (BioG) and intestine (BioGI) compartments by 15.5% for BioG-Cd, 39.2% for BioGI-Cd, 28.8% for BioG-As, and 45.0% for BioGI-As, thus reducing the human health risk of Cd and As from soil ingestion. The use of oyster shell waste to produce Ca-humic complex as a soil amendment is a practical means of achieving dual agronomic and environmental benefits, from the remediation of soil contamination to the safe disposal of shell waste.

Keywords: cadmium; arsenic; soil contamination; bioavailability; unified bio-accessibility method



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1. Introduction

Soil contamination of heavy metal(loid)s has become a problem in many parts of the world. In China, for example, a nation-wide soil survey found that cadmium (Cd) concentrations in 7% of sampling sites exceeded the national environmental standard, and the number for arsenic (As) was 2.7% [1]. Remediation of contaminated soils, particularly those with a low pH, is desirable to reduce the bioavailability and uptake of Cd and As by cereals and vegetables for safe consumption. A commonly used technology for soil remediation is immobilization [2]. It reduces the chemical activity and bioavailability of heavy metal(loid)s via chemical reactions with soil amendments such as minerals, humic substances, and biowaste [3,4]. Lime is probably the most frequently used amendment for metal immobilization in acidic soils.

Although lime is a useful amendment for alleviating soil acidity and immobilizing heavy metal(loid)s, its production is an environmentally unfriendly process, involving the excavation of limestone and its calcination at 900–1100 °C. The former destroys the natural landscape, and the latter consumes much energy and releases the greenhouse gas carbon dioxide. It would be better to reduce environmental impact by producing lime from shell waste at a lower calcination temperature.

Shells are natural materials, the basic unit of which is a nanocomposite consisting of brick-like layers of calcium carbonate (ca. 96% by weight) [5] and thin layers of protein as organic glue. Shells are produced in large quantities in China as waste from aquaculture.

For example, 4.57 million tons of oyster was produced in 2015 [6]. As a result, a large amount of oyster shell (OS) waste has been piled on the coast, producing odor and breeding insects. The conversion of OS to lime for use as a soil amendment provides a means of reversing calcium loss in acidic soils and helping aquaculture by eliminating the waste problem. Nature is amazing in that it not only builds brick-like layers of calcium carbonate from seawater but also provides a nanocomposite structure, the organic moiety of which can be removed via calcination at a lower temperature (400–500 °C) than that of calcium carbonate decomposition [7]. Removing organic moiety creates structural voids in OS, making the shell conversion to lime easier and faster than that from limestone to lime.

This research aimed to investigate the effectiveness of OS-converted lime combined with a leonardite-derived humic substance in immobilizing Cd and As in soil, reducing their uptake by a commonly consumed vegetable and lowering their bio-accessibility. Cd and As are two toxic and carcinogenic contaminants with opposite chemical behaviors in soil, and simultaneously immobilizing them is a big challenge. Adding humic substance to lime produces dual benefits of forming a Ca-humic complex with additional adsorptive sites for Cd and As and reducing the destructive effect of lime on soil structure.

2. Materials and Methods

2.1. Preparation of Ca-Humic Complex

The soluble humic substance was extracted from a leonardite and characterized for its physical and chemical properties [8]. Briefly, a leonardite powder was first added to a centrifuge tube with 0.1 M KOH solution at a 1:40 (g: mL) ratio. After 2 h shaking at 25 °C and 10 min centrifugation at 3000 r/min, the supernatant was collected for use as a soluble humic substance (HS).

Oyster shell (OS) was collected and calcined, and its physical, chemical, and mineralogical properties were characterized, as detailed elsewhere [8]. Briefly, OS (<2 mm) was calcined at 800 °C for 4 h in a muffle furnace and then ground to pass through a 60-mesh nylon sieve (referred to as OS₈₀₀).

The Ca-humic complex was prepared by adding 40 mL of HS to a centrifuge tube with 0.800 g OS₈₀₀, shaken at 300 r/min for 4 h at 25 °C, and centrifuged at 4000 r/min for 15 min. After the supernatant was poured off, the formed Ca-humic complex (i.e., the residue in the tube) was washed with deionized water three times, oven-dried at 60 °C, and ground to pass through a 60-mesh nylon sieve for use in this study. The complex was analyzed for its pH by a pH meter (Mettler Toledo Five Easy Plus, Zürich, Switzerland), elemental compositions by using an elemental analyzer (Elementar Micro cube, Hesse, Germany), and ash content by heating in a muffle furnace at 800 °C for 4 h. A Fourier transform infrared (FTIR) spectrometer (Thermo Fisher Nicolet iS50, Waltham, MA, USA) was used to record the spectra of the Ca-humic complex and its products from the reaction of the complex (20 mg) with 40 mL of Cd(NO₃)₂·4H₂O (136 mg Cd/L) or Na₃AsO₄·12H₂O (100 mg As/L), with a resolution of 2 cm⁻¹ over the 400–4000 cm⁻¹ wavenumber region. The contents of carboxyl and phenolic groups of the Ca-humic complex were determined by the method of the International Humic Substances Society (IHSS) [9].

2.2. Soil Sample

A topsoil (0–20 cm) sample was collected following an S-route sampling from a vegetable farm along the lower reach of the Xijiang River (West River) in Foshan City, Guangdong Province (22°58' N, 112°52' E). It had a pH of 6.03, total Cd of 5.23 mg/kg, total As of 9.88 mg/kg, organic carbon of 8.56 g/kg, and sand (2–0.02 mm), silt (0.02–0.002 mm), and clay (<0.002 mm) contents of 41.73%, 46.25%, and 12.02%, respectively [7]. The soil is classified as Entisols in the USDA Soil Taxonomy and Fluvisols in the FAO World Reference Base for Soil Resources.

2.3. Pot Experiment

The bioavailability of soil Cd and As to plants was assessed by a pot experiment using a popular vegetable, bok choy or pak choy (*Brassica rapa subsp. chinensis*), as an indicator plant. The Ca-humic complex was added to and mixed well with the soil at 0, 0.5, 1.0, 2.0, and 5.0% dosages in triplicate. After the germination of bok choy seeds, three plants were kept for growth in each pot in an artificial climate chamber (25 °C, 55% relative humidity) for 30 d, during which soil moisture content was maintained at 70% of the field capacity. In the end, the bok choy was harvested, oven-dried, and digested with nitric acid [10] to analyze Cd and As contents by inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC II, PerkinElmer, Waltham, MA, USA).

2.4. Bio-Accessibility Evaluation of Cd and As in Soil

The unified bio-accessibility method (UBM) was followed to estimate the bio-accessibility of Cd and As in soil to human beings and assess the human health risk of Cd and As via soil ingestion. The UBM includes in vitro tests of the simulated stomach and intestine compartments [11]. Briefly, for the simulated stomach test, soil (1 g) was added to centrifuge tubes with a simulated gastric fluid (22.5 mL), and the pH of the mixture was adjusted to 1.1 ± 0.2 by adding 10 M NaOH or 37% HCl. The tubes were then placed on a rotary shaker (55 r/min) for 1 h at 37 ± 2 °C and centrifuged at 4000 rpm for 20 min. The supernatant was filtered through 0.45 µm and acidified with 1 drop of 1% HNO₃ for analyses of Cd and As by ICP-MS. In the simulated intestine test, soil (1 g) was first added to centrifuge tubes with a simulated gastric fluid (22.5 mL), and the pH of the mixture was adjusted to 1.1 ± 0.2 . After the tubes were shaken at 55 r/min for 1 h at 37 ± 2 °C, 60 mL of simulated duodenal phase fluid was added to the tubes and shaken for 4 h. In the end, a portion of the mixture was centrifuged at 4000 rpm for 20 min, filtered through 0.45 µm, and acidified with 1 drop of 1% HNO₃ for analyses of Cd and As by ICP-MS. The above experiments were conducted in triplicate, with blanks and a certified reference soil for quality assurance, with a recovery rate of >95%.

3. Results

3.1. Properties of Calcined Oyster Shell and Ca-Humic Complex

As reported earlier [7], the mineral composition of the finished product (OS₈₀₀) from OS calcination at 800 °C for 4 h was calcium oxide, with a pH of 12.75, total Cd content of 0.026 mg/kg, and total As content of 0.708 mg/kg.

The basic properties of the Ca-humic complex are shown in Table 1. With abundant carboxyl and phenolic hydroxyl groups, the complex has an excellent potential to bind Cd and As, thus underpinning its effective use in soil remediation.

Table 1. Physical and chemical properties of the Ca-humic complex.

| C | H | O | N | S | Ash | pH | Phenolic-OH | –COOH | Total Cd | Total As |
|--------------|-------------|--------------|-------------|-------------|--------------|-------|-------------|-------------|---------------|--------------|
| | | | % | | | - | mol/kg | | mg/kg | |
| 30.20 ± 0.21 | 3.44 ± 0.10 | 34.16 ± 1.21 | 0.56 ± 0.05 | 0.84 ± 0.08 | 30.80 ± 1.23 | 11.08 | 0.82 ± 0.15 | 2.34 ± 0.29 | 0.035 ± 0.008 | 1.26 ± 0.084 |

The reaction of the Ca-humic complex with Cd or As was indicated by FTIR spectra (Figure 1). In agreement with the high contents of carboxyl and phenolic hydroxyl groups (Table 1) determined by the titration method of IHSS, the Ca-humic complex contained a large number of oxygen-containing functional groups, with the ability to donate electron pairs and to bind Cd and As [12]. The peak at 3211 cm⁻¹ was hydrogen-bonded O–H stretching [13], and it played a role in binding Cd and As. The peak around 1544 and 1387 cm⁻¹ was due to the asymmetric and symmetric stretching of COO⁻ carboxylic acid [14,15]. The separation between these two peaks shrank from 162 to 157 and 141 cm⁻¹ after reacting with As or Cd. Furthermore, the intensity of the symmetric stretching visually increased. Thus, carboxyl groups were involved in forming chelate with Cd and As [15,16]. Besides, the peak around 870 cm⁻¹ was ascribed to the C–H bending of

aromatic compounds. The enhancement and broadening of this peak after reacting with As or Cd indicated the cation– π interaction. For Cd, it can be regarded as the positive Cd^{2+} directly bonding with the aromatic π electron donor in the HA, i.e., the polycondensed aromatic structures, via a type of non-covalent interaction [16]. In contrast, the As anionic species (AsO_4^{3-} or AsO_3^{3-}) might first bridge with Fe^{3+} in soil solution and then form ternary complexes with HS [17]. In summary, Cd and As could bind to the complex via hydrogen bond interaction, carboxyl chelation, and cation– π interaction.

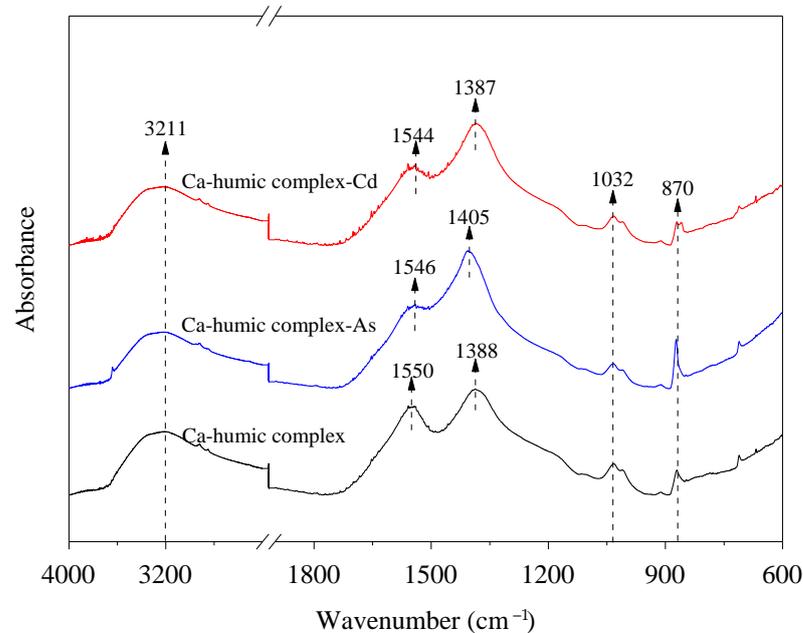


Figure 1. FTIR spectra of the Ca-humic complex before and after adsorbing Cd and As.

3.2. Ca-Humic Complex Reduced Vegetable Uptake of Cd and As

Figure 2 and Table 2 show bok choy growth in response to the use of the Ca-humic complex as a soil amendment. A dose of 0.5%, 1.0%, or 2.0% had little effect on the above-ground biomass of bok choy, whereas a 5.0% dose severely inhibited its growth, probably due to the adverse impact of a significant increase in soil pH. The Ca-humic complex showed its ability to reduce the bioavailability of soil Cd and As to plants, as evidenced by changes in the Cd and As contents in the edible part of bok choy. Compared with the control (CK), a 2.0% dose of Ca-humic complex reduced Cd and As contents by 98% and 71%, respectively. The contents were close to their limits ($Cd \leq 0.05$ mg/kg, $As \leq 0.5$ mg/kg) in the Chinese National Food Standard (GB 2762-2017). Furthermore, the complex itself had low Cd (0.035 mg/kg) and As (1.269 mg/kg) contents, making its use as a soil amendment safe.

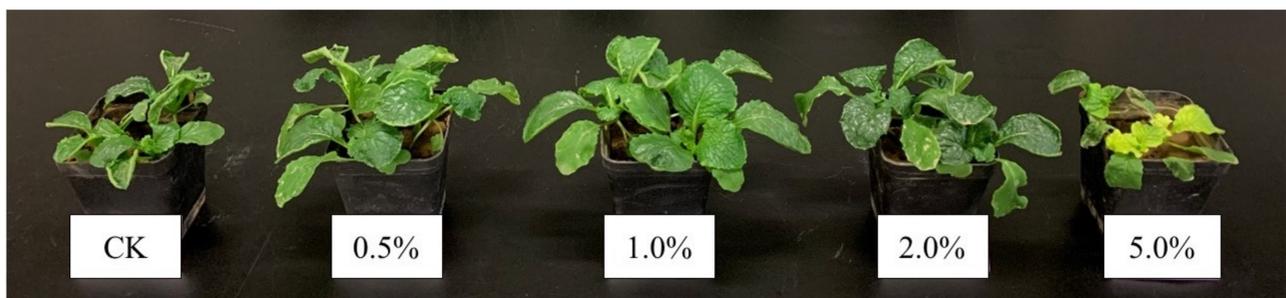


Figure 2. Effects of Ca-humic complex dosages on bok choy growth.

Table 2. Ca-humic complex affects the above-ground biomass and Cd and As contents of the vegetable bok choy.

| Dose | | CK (0%) | 0.5% | 1.0% | 2.0% | 5.0% |
|------------------------------|------------|---------------|---------------|---------------|---------------|---------------|
| Soil pH | At sowing | 6.03 | 6.78 | 6.99 | 7.86 | 8.56 |
| | At harvest | 6.01 | 6.03 | 7.58 | 7.31 | 7.89 |
| Above-ground biomass (g/pot) | | 0.46 ± 0.12 a | 0.45 ± 0.06 a | 0.47 ± 0.02 a | 0.46 ± 0.02 a | 0.32 ± 0.01 b |
| Cd (mg/kg) | | 2.80 ± 0.73 a | 0.89 ± 0.18 b | 0.10 ± 0.01 c | 0.05 ± 0.00 c | 0.05 ± 0.00 c |
| As (mg/kg) | | 1.73 ± 0.04 a | 1.08 ± 0.22 b | 0.31 ± 0.09 c | 0.51 ± 0.05 c | 0.49 ± 0.13 c |

Different lowercase letters indicate a significant difference between doses ($p < 0.05$).

3.3. Ca-Humic Complex Reduced Bio-Accessibility of Soil Cd and As

Table 3 shows the bio-accessibility of Cd and As at simulated gastric (*BioG*) and intestinal (*BioGI*) compartments. Specifically, the feeding ranges at the gastric stage were 64.31–80.66% for Cd and 27.89–46.52% for As; at the intestine stage, the feeding ranges were 22.92–52.06% for Cd and 22.10–54.32% for As. A percentage of less than 100 indicates the partial bio-accessibility of soil Cd and As in the simulated stomach and intestine compartments. With the increased dosage of Ca-humic complex, *BioG*-Cd, *BioG*-As, *BioGI*-Cd, and *BioGI*-As all decreased, suggesting that the Ca-humic complex would reduce the human health risk of soil Cd and As should the soil be unintentionally ingested.

Table 3. Ca-humic complex lowers the bio-accessibility of soil Cd and As at simulated gastric (*BioG*) and intestinal (*BioGI*) compartments.

| Dose | <i>BioG</i> -Cd (%) | <i>BioGI</i> -Cd (%) | <i>BioG</i> -As (%) | <i>BioGI</i> -As (%) |
|-----------|---------------------|----------------------|---------------------|----------------------|
| 0.0% (CK) | 80.66 ± 2.13 | 52.06 ± 2.37 | 46.52 ± 3.80 | 54.32 ± 1.55 |
| 0.5% | 75.78 ± 0.46 | 44.99 ± 2.50 | 45.85 ± 3.24 | 43.05 ± 4.90 |
| 1.0% | 69.61 ± 0.87 | 35.39 ± 1.84 | 37.63 ± 1.13 | 36.51 ± 1.71 |
| 2.0% | 68.16 ± 2.91 | 31.64 ± 2.67 | 33.11 ± 0.20 | 29.88 ± 1.47 |
| 5.0% | 64.31 ± 1.32 | 22.92 ± 0.19 | 27.89 ± 0.30 | 22.10 ± 0.20 |

4. Discussion

4.1. Ca-Humic Complex as a Soil Amendment Reduced the Phytoavailability and Bio-Accessibility of Cd and As in Soil

The FTIR spectra (Figure 1) show that the carboxyl and phenolic hydroxyl groups from the organic moiety of the Ca-humic complex played a role in binding Cd and As, thus reducing their reactivity and uptake by the vegetable. Besides, the inorganic moiety of the complex could also adsorb Cd and As. The complex had a high ash content (30.8%), including added OS_{800} (CaO) and the mineral residue of humic substance. CaO reacted with water to become $Ca(OH)_2$ with a solubility of 1.73 g/L (20 °C) and K_{sp} of 5.5×10^{-6} . The dissolution and ionization of $Ca(OH)_2$ resulted in a high pH and abundant Ca^{2+} . The former would precipitate Cd^{2+} , and the latter can react with AsO_4^{3-} to form insoluble $Ca_3(AsO_4)_2$ at high pH [18]. The very low K_{sp} of $10^{-21.14}$ at 25 °C [19] of $Ca_3(AsO_4)_2 \cdot 3H_2O$ contributed to the continuous removal of As by Ca. Therefore, both the organic and inorganic moieties of the Ca-humic complex contributed to the binding of Cd and As to the complex via adsorption and precipitation, thus reducing the mobility of Cd and As and their uptake by bok choy.

The above analysis also helps explain the decrease in the bio-accessibility of soil Cd and As. After mixing with the soil, the Ca-humic complex provided additional binding sites for Cd and As, making them less soluble in the simulated gastric and intestinal environment. Overall, Cd had a higher bio-accessibility in the gastric compartment than in the intestinal one because the pH of the former was much lower than the latter. This difference in bio-accessibility between the two compartments agrees with reports by other researchers [20,21]. The increased intestinal pH would cause precipitation of dissolved Cd and its adsorption onto Fe/Al/Mn hydroxides newly formed in the intestinal compartment.

In comparison with Cd, the difference in the bio-accessibility of As between gastric and intestinal compartments was smaller but still influenced by the pH increase from the gastric to the intestinal environment because the Fe/Al/Mn hydroxides formed at the intestinal compartment could also adsorb As.

As the dose of Ca-humic complex increased, the bio-accessibility of soil Cd and As in gastric and intestinal compartments decreased. Together with the reduced vegetable uptake of Cd and As, this result proves that the Ca-humic complex is an effective soil amendment to reduce the risk of Cd and As in soil to human health via the food chain and soil ingestion pathways.

4.2. Agronomic Benefits and Environmental Significance of Using Shell Waste as a Soil Amendment

The weathering of rocks and minerals releases solutes and facilitates the formation of soil, which enables life on Earth [22,23]. Indeed, weathering supplies most of the base cation nutrients (e.g., Ca, K) to plants. Acidic soils (e.g., ferrosols) in tropical and sub-tropical regions in an advanced weathering stage are inadequate in Ca. Lime application is often required to remediate soil acidity and associated problems such as heavy-metal contamination. Mining limestone for lime, however, is environmentally unfriendly. Furthermore, limestone might contain heavy metals that are difficult to remove. For use as a soil amendment to improve soil productivity and remediate contaminated soil, it would be easier to accept lime from seafood sources (e.g., crustaceans and mollusks) than from limestone.

Biomineralization is the process by which living organisms produce minerals. The most common biominerals are the phosphate and carbonate salts of calcium that form structural features (e.g., seashells and bones in mammals). Shells of crustaceans and mollusks could be added to acidic soil to partially reverse the loss of Ca from land to the ocean, and the large number of seashells available for conversion to lime makes it possible. For example, about 9.38 million tons of crustaceans (e.g., crab, shrimp, and lobster) and 17.51 million tons of dominant molluscan species (e.g., pipi, cockles, mussels, oysters, and scallops) were produced in 2018 globally [24], and China was one of the largest producers.

The Pacific oyster is used here as an example to estimate the amount of Ca in shells. The yield of meat from Pacific oysters typically ranges from 5 to 14% [25]. In other words, the shell accounts for 86–95%. By weight, ca. 96% of the shell is calcium carbonate [5]. Thus, 33–36% of the oyster weight would be Ca, and 17.51 million tons of molluscan species contain about 5.8 million tons of Ca. In comparison, the annual (dissolved) Ca flux from the largest river in China (Yangtze River) to the ocean was estimated at 67×10^{10} mol/yr or 2.68×10^7 tons/yr [26]. Therefore, Ca in shells is equivalent to a significant percentage of the dissolved Ca migration from land to the ocean.

Although human beings have not found a cost-effective technology to recover Ca from the sea, nature has been performing this function since time immemorial. By capitalizing on the natural nanotechnology of crustaceans and mollusks in building shells, human beings could partly reverse the loss of Ca from tropical and subtropical land to sea by using shells as a soil amendment. Shell-to-lime conversion would have agronomic and environmental benefits. Besides benefiting the remediation of soil acidity and soil contamination, shell conversion to lime helps solve the nasty smell and public health problems from untreated shell waste, reduces disposal costs, and enhances the sustainability of aquaculture.

5. Conclusions

Oyster shells were converted to lime by heating at 800 °C for 4 h. They reacted with leonardite-derived humic substance to form a Ca-humic complex. The complex was insoluble, and its pH was lower than lime. Thus, it is safer to handle in the field and less destructive to soil structure than lime. The complex contributes organic matter to the soil and adsorbs Cd and As. As a soil amendment at a 2% dose, the Ca-humic complex reduced Cd and As contents in the edible part of bok choy by 98% and 71%, respectively, making the vegetable safe for consumption. The complex also reduced the bio-accessibility of soil Cd and As in the simulated gastric and intestine environment. A 2% dose lowered

Cd and As bio-accessibility in the gastric compartment by 15% and 29%, respectively, and in the intestine compartment by 40% and 45%, respectively. Thus, the Ca-humic complex decreased the human health risk of Cd and As in soil from both food chain and soil ingestion pathways.

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