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Evaluation of the effects of mandarin (*Citrus reticulata*) by-products containing citric acid on immobilization of cadmium in arable soils

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Abstract

In a pilot study, we observed cadmium (Cd) immobilization with citric acid (CA) and suggested that mandarin by-products (MB), which contain CA at ca. 1.65%, can be used as soil amendments that reduce Cd bioavailability. In the present study, we (1) elucidated mechanisms of Cd immobilization by CA and (2) evaluated the use of MB as a soil amendment for Cd immobilization. In Experiment 1, CA was mixed with Cd contaminated soil at 0 and 3.5 mmol kg⁻¹. We then added MB to Cd-spiked soil at 10, 20, and 40 g kg⁻¹. Addition of CA decreased F2 (surface adsorbed Cd fraction) contents by 2.64 mg kg⁻¹ compared with the control but was associated with increases in Cd fractions F1 (bioavailable Cd fraction) and F5 (residual Cd fraction) of 1.04 and 1.49 mg kg⁻¹, respectively. Addition of CA enhanced the concentration of fraction F5, likely reflecting Cd precipitation from soil solutions with increased HCO₃⁻ concentrations. However, although this treatment immobilized Cd, it also led to increasing residual and bioavailable Cd fractions. Unlike CA treatments, MB increased non-bioavailable Cd fractions without increasing the bioavailable Cd fraction. Moreover, at 40 g kg⁻¹, MB decreased F1 contents by 8% compared with the control, but increased F2, F3, and F5 contents by 3.6%, 0.7%, and 4.5%, respectively. Cd may be immobilized by MB through H_xCO₃⁻ mediated precipitation as CdCO₃ following decomposition of CA and concomitant increases in the negative charge of soil due to the organic matter in MB. MB also improved the chemical properties of soils, with increased nutrient concentrations and cation exchange capacities.

Keywords: Cadmium, Carbonate, Citric acid, Immobilization, Mandarin byproduct

Introduction

Recently, the production of cadmium (Cd) containing compounds has dramatically increased due to rapid industrialization [1]. The annual world total production of Cd in 2018 was 26,000 tons [2]. Most of the world's primary Cd metal was produced in Asia, and the leading global producers were China, the Republic of Korea, and Japan. The wide use of Cd containing materials in industry results in irreversible environmental exposures to Cd, which finally ends up in the soil [3]. Due to its high toxicity in the food chain, the presence of Cd in arable soils

could be hazardous to humans, even at low levels [4, 5]. Cadmium poisoning causes itai-itai disease, and chronic exposure to Cd can result in nephrotoxicity in humans, mainly reflecting the development of abnormalities in tubular re-absorption [6, 7]. According to previous studies of heavy metals in agricultural soils, Cd was ranked among the most harmful heavy metals of major heavy metal contaminants globally [8].

Phytoremediation is an optimal technology for restoring Cd contaminated arable soils, and is economically and environmentally appropriate. Over several decades, many studies demonstrate phytoremediation of Cd contaminated arable soils [9–17]. In some of these, chelating agents that increase the extractability of Cd were used to boost uptake of this metal by hyperaccumulators [9, 11, 12, 15]. Chelating agents such as citric

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acid (CA), diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), ethylenebis(oxyethylenenitrilo)-tetraacetic acid (EGTA), maleic acid, and *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid (CDTA), form metal–ligand complexes with Cd²⁺ that are more readily taken up by plant roots than the free ion Cd²⁺ [18, 19]. Therefore, the application of chelating agents to conventional phytoremediation techniques in Cd contaminated soil has received keen interest. Although these techniques are highly effective, we observed Cd immobilization with CA in our recent pilot study [20]. We reported that concentrations of non-bioavailable Cd fractions increased following addition of CA, but we did not precisely demonstrate the related mechanism of Cd immobilization with CA.

Mandarin orchards cover 20,140 ha in the Jeju island region of South Korea, where approximately 576,722 Mg of mandarins were produced in 2017 [21]. About 51,000 ton of mandarin byproduct (MB) is dumped annually in Jeju [22], and this byproduct generates potent odors, leads to destruction of the natural landscape, and causes various environmental problems. Therefore, utilization of MB has become an imminent issue in Jeju. Yet, because biosynthetic sources offer a plentiful supply of organic acids, recycling of MB is limited. Mandarin is a representative citrus fruit that contains very high quantities of organic acid. Its scientific name *Citrus reticulata* implies

that CA is the major organic acid component of mandarins. Although many studies show uses of MB in various fields, use as a soil amendment for remediating Cd in arable soil has not been reported previously. Because MB contains CA at ca. 1.65%, we investigated its effects as a soil amendment to reduce Cd bioavailability in soils. Specifically, we (1) elucidated the mechanism of Cd immobilization by CA and (2) evaluated the feasibility of MB as a soil amendment for Cd immobilization.

Materials and methods

Soil characteristics and Cd spiking

Upland soil was collected from the top 10 cm at an experimental field of Pusan National University (128°43' N and 35°30' E), Republic of Korea. Soil samples were air-dried and ground through a 2-mm sieve. Selected physical and chemical properties of the soil samples are summarized in Table 1. Soils were spiked with CdCl₂·2.5H₂O to give a total Cd concentration of 20 mg Cd kg⁻¹ [23]. Subsequently, 20 kg arable soil samples were added to 15 L of distilled water containing 0.813 g of CdCl₂·2.5H₂O to achieve a Cd concentration of 20 mg kg⁻¹. The resulting mixtures were subjected to an aging period, because solutions of CdCl₂·2.5H₂O contain mostly soluble forms of Cd. After Cd-spiked soils were completely dried, 15 L aliquots of distilled water were spread and mixed thoroughly with soils using a plastic spatula. Soils were then

Table 1 Selected properties of soil and mandarin by-product

Studied material	Items	Concentration
Soil	pH (1:5 with H ₂ O)	6.71
	Total nitrogen (g kg ⁻¹)	1.05
	Organic matter (g kg ⁻¹)	17.6
	Available phosphorus (mg kg ⁻¹)	142
	Cation exchange capacity (cmol _c kg ⁻¹)	6.85
	Exchangeable cation (cmol _c kg ⁻¹)	
	Ca	4.76
	K	0.42
	Mg	0.93
	Na	0.38
	Total concentration of cadmium (mg kg ⁻¹)	trace
	Bulk density (g m ⁻³)	1.41
	Soil texture	Sandy loam
	Clay (%)	14.9
	Silt (%)	25.7
Sand (%)	59.4	
Mandarin by-product (<i>Citrus reticulata</i>)	pH (1:5 with H ₂ O)	4.02
	Total nitrogen (g kg ⁻¹)	22.9
	Organic matter (g kg ⁻¹)	85.1
	Total concentration of cadmium (mg kg ⁻¹)	trace
	Citric acid (% wt ⁻¹)	1.65

air-dried and the aging process was repeated three times more over 70 days.

MB properties

MB was provided by the mandarin processing plant at Seogwipo-si (126°66' N and 33°32' E), Republic of Korea. MB was air-dried and crushed using a blender and was then filtered through a 0.84-mm sieve for analyzes of chemical properties (Table 1).

Incubation tests

Experiment 1: effects of CA on Cd immobilization

CA was added to 200 g samples of pretreated soils to concentrations of 0 and 3.5 mmol kg⁻¹ in 160 mL plastic beakers. Soil mixtures were then incubated in the dark at 25 °C for 6 weeks, wetted to 70% of corresponding pore volumes, and weighed every 2 days to maintain water contents. Soil mixtures were then randomly placed in an incubator and experiments were conducted with four replications.

Experiment 2: feasibility of MB as a soil amendment that immobilizes Cd

MB was added to 200 g samples of pretreated soil to concentrations of 0, 10, 20, and 40 g kg⁻¹ in 160 mL plastic beakers. Experiment 2 was conducted under the same conditions as described above for Experiment 1.

After the incubation periods in Experiments 1 and 2, soils were dried and sieved through < 2 mm filters for further extraction and chemical analysis.

Chemical analysis

Soil pH (1:5, H₂O) was measured using a pH meter (Starter 3000, Ohaus, USA). Negative charges of soils were analyzed by measuring K⁺ retention as described by Macrano-Martinez and McBride [24] with some modifications. Dissolved organic carbon (DOC) was quantified using a total organic carbon (TOC) analyzer (Model TOC-VCPN; Shimadzu, Japan).

After 6-weeks incubation, soils were air-dried and digested with aqua regia (3:1, HCl:HNO₃) for analysis of total Cd concentrations. Cd was then sequentially extracted from soils using a modified sequential Cd extraction method [25, 26]. After Cd fractionation, suspensions were centrifuged at 605×g for 15 min to separate thoroughly extractants from soils. The remaining supernatants were then filtered through 0.45 μm cellulose acetate membrane filters and Cd contents were quantified using atomic absorption spectroscopy (AAS; Perkin Elmer Model 3300, Shelton, Connecticut, USA).

The present five modified Cd fractions differ in bio-availability, binding strength, and chemical form [27, 28]. In particular, Cd fraction 1 (F1) is a bioavailable

fraction that contains water soluble, exchangeable, and weak-acidic extractable forms of Cd. Cd fraction 2 (F2) was the surface adsorption fraction and contained the immobilized Cd form from soils. F2 is not readily taken up by plants because it is tightly bound to surfaces of soil colloids by specific adsorption or weak carbonated bonds. Cadmium fraction 3 (F3) is strongly complexed by organic matter. Cadmium fraction 4 (F4) is the reducible fraction that is associated with iron (Fe) and manganese (Mn) oxides. Cadmium fraction 5 (F5) is the residual form and is mostly present as Cd minerals, or is fixed crystal lattices [25–28].

Modeling of saturation indexes for Cd minerals

The Visual MINTEQ (version 2.23) chemical equilibrium speciation program was used to calculate saturation indexes for Cd minerals. Soil pH and ionic strength were fixed at the measured value, and in equilibrium with atmospheric CO₂ were assumed to be 0.003 atm. Calculations were performed with the input parameters for modeling pH, ion strength, cations (Ca²⁺, Cd²⁺, Fe²⁺, Fe³⁺, K⁺, Mg²⁺, Na⁺, Ni²⁺, NH₄⁺, Pb²⁺, Si⁴⁺, and Zn²⁺), anions (Cl⁻, CO₃²⁻, NO₃⁻, PO₄³⁻, and SO₄²⁻), and DOC at 25 °C. Soil pH was measured using a pH meter. Cations (Ca²⁺, Cd²⁺, Fe²⁺, Fe³⁺, K⁺, Mg²⁺, Na⁺, Ni²⁺, Pb²⁺, Si⁴⁺, and Zn²⁺) were quantified using an inductively coupled plasma mass spectrometer (ICP-MS; Thermo Scientific iCAP Q, Bremen, Germany). Ammonium concentrations were measured using a colorimetric method [29]. Cl⁻, NO₃⁻, PO₄³⁻, and SO₄²⁻ contents were quantified using an ion chromatograph (IC; Dionex Corporation Model ICS-2000 IC, USA). Concentrations of bicarbonate (HCO₃⁻) were measured using a potentiometric titration method [30].

Production of carbonate species from CA decomposition by soil microbes

In vitro tests were designed to determine the production of carbonate species (H₂CO₃, HCO₃⁻, and CO₃²⁻) from the decomposition of MB including CA by soil microbes. Briefly, 500-ml aliquots of distilled water were added to 50 g samples of Cd free soil and the soil was incubated for 48 h. Soil slurry was then filtered through 0.22 μm cellulose acetate membranes. The filtered solutions were then inoculated into 200 ml of BR medium containing 1.0 g l⁻¹; ammonium sulfate, 0.1 g l⁻¹ dipotassium hydrogen phosphate, 3.0 g l⁻¹ potassium dihydrogen phosphate, and 0.2 g l⁻¹ magnesium sulfate heptahydrate in a 500-ml Erlenmeyer flask. CA and MB were added to BR medium at 0 and 10 g l⁻¹. BR mediums were then incubated at 36 °C for 120 h in a shaking incubator. After incubation, media were centrifuged, and the supernatants were discarded. To measure concentrations of all

carbonate species (H_2CO_3 , HCO_3^- , and CO_3^{2-}), sample pH was adjusted at 8.34 using 0.005 M NaOH and HCl, because all carbonate species are converted to HCO_3^- at pH 8.34 [31]. Once sample pH values were stable, carbonates in suspensions were determined by potentiometric titrations [30].

Statistical analysis

Statistical analyzes were performed using Statistix version 9.0. Mean values of Cd fractions, soil pH, and negative charges were compared using a pairwise differences method. Mean effects were separated using least significance differences when the appropriate F test was significant ($p < 0.05$).

Results and discussion

To identify the availability of heavy metals in contaminated soils, we performed a sequential extraction method for heavy metals as described in previous studies [20, 29, 32, 33]. Among the present fractions, F1 contained the most bioavailable Cd. Yet, F2 can be readily converted to F1 following changes in soil factors such as pH, organic matter, mineralogy, and anions and cations on soil surfaces that are known to influence soil surface complexation [34, 35]. Hence, F2 is thought to be the temporarily stable form of Cd fractions. In contrast, F5 is considered a stable form that is not readily changed into bioavailable forms under natural soil conditions. In this study, we assumed that the three Cd fractions F1, F2, and F5 are keys to evaluations of the effectiveness of CA and MB on the immobilization of Cd.

Effects of CA on Cd immobilization

F1 concentrations significantly increased with the addition of CA compared with those of control experiments (Table 2). Hence, the bioavailability of Cd may be artificially increased by adding synthetic chelating agents such as CA. Similarly, other researcher report increases in exchangeable Cd fractions in soils treated with CA [11, 15, 18, 36]. Formation equilibrium constants for complex formation between Cd^{2+} and chelating agents are sufficiently high to extract bound Cd from soil matrixes. For

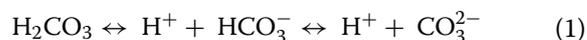
example, a log K_f of 4.54 was calculated for complexation of Cd^{2+} with CA, where

$$K_f = [Cd - CA^{3-}] / [Cd^{2+}] [CA^{3-}]$$

for the predominant complexed form of $[Cd - CA^{3-}]$ [37]. The addition of CA decreased the Cd contents of F2 by 2.64 mg kg⁻¹ compared with the control and led to corresponding increases in Cd contents of 1.04 mg kg⁻¹ and 1.49 mg kg⁻¹ in F1 and F5 fractions, respectively (Table 2). These observations suggest that the Cd fraction was extracted and transformed to a bioavailable fraction and a residual fraction following application of CA. F5 associated with Cd precipitates and fixed Cd in crystal lattices markedly increased with CA. No previous reports show the effects of CA on increasing residual Cd fractions, because chelating agents such as CA are well known to increase Cd phytoextractability. Hence, in this study, we investigated the mechanisms by which F5 contents increased with CA addition. We hypothesized that (1) CA is decomposed by soil microorganisms, leading to the production of various carbonate species (H_2CO_3 , HCO_3^- , and CO_3^{2-} ; H_xCO_3), and (2) that increases in these carbonates promote Cd^{2+} precipitation as otavite ($CdCO_3$), leading to increased F5 concentrations.

To test the first hypothesis, H_xCO_3 concentrations from CA were measured in BR medium. CA significantly increased H_xCO_3 concentrations to about 11 fold that in controls (Fig. 1). This result was similar to observations by Bruce [38], who reported that 29–42% of carbonic acid was produced from the decomposition of CA by *Bacillus* sp.

To test the second hypothesis, we assumed that Cd^{2+} reacts with H_xCO_3 to form $CdCO_3$, which was present at increased concentrations in fraction F5. Under these conditions, H_xCO_3 is dissociated in soil solutions according to the following reaction [31, 39]:



The first and second dissociation constants of carbonate species are 6.36 and 10.33, respectively. Hence, bicarbonate ion (HCO_3^-) is the dominant carbonate species in our studied soil samples, which had pH=6.71 (Table 1).

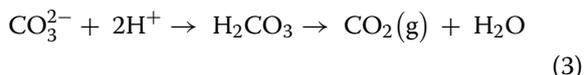
Table 2 Concentrations of Cd fractions in soils amended with and without citric acid (CA) after a 6-week incubation at 25 °C; differing superscript letters in columns indicate significant differences; $p = 0.05$

Treatment	Cadmium fraction (mg kg ⁻¹)							
	F1	F2	F3	F4	F5	Sum	Total	Recovery rate (%)
Control	4.62 ^b	11.8 ^a	2.75 ^a	0.94 ^a	0.75 ^b	20.9	20.4	102
CA	5.66 ^a	9.17 ^b	2.72 ^a	0.98 ^a	2.24 ^a	20.8	20.2	103

Therefore, in equilibrated solutions, CdCO₃ might be predominantly formed by the following reaction [40]:



After 6-weeks incubation with CA, soil pH increased slightly (Table 3), even though according to Eq. 2, soil pH should have decreased with CA. This discrepancy may be due to increased concentrations of carbonate ion (CO₃²⁻) from the decomposition of CA. CO₃²⁻ increases soil pH according to the following reaction [31];



We assumed that Cd²⁺ and HCO₃⁻ would be solubilized and supersaturated in solutions, allowing the formation of CdCO₃. Theoretically, ions can precipitate from solutions when the ion activity product (IAP) is higher than the mineral solubility product (K_{sp}). The saturation index [SI=log(IAP/K_{sp})] is a practical indicator that can be used to determine whether the solution is in equilibrium with given inorganic mineral parameters, and when SI=0, the soil solution is in equilibrium with the given Cd minerals. When SI>0, the solution is supersaturated with respect to given Cd minerals, and when this value is <0 the soil solution is undersaturated relative

to Cd minerals. Chemical immobilization treatments could change cadmium solubility and increase stability by precipitation of Cd minerals [41]. Especially, three Cd minerals (CdCO₃, Cd₃(PO₄)₂, and Cd(OH)₂) may form but other Cd minerals (CdCl₂ and CdSO₄) is too soluble to form in the natural soil environment [42]. To determine whether Cd²⁺ precipitated as CdCO₃, Cd₃(PO₄)₂, and Cd(OH)₂, SIs of these minerals were calculated using a chemical speciation program. The SI for all Cd minerals showed that soil suspensions of control and CA treatments were undersaturated relative to Cd₃(PO₄)₂ and Cd(OH)₂ (Table 3). However, the SI for CdCO₃ was higher than 0 in the presence of CA, implying that Cd²⁺ and HCO₃⁻ activities in soil solutions with CA were high enough to form CdCO₃.

Addition of CA enhanced concentrations of F5, potentially reflecting Cd precipitation induced by increases in HCO₃⁻ concentrations in soil solutions. These results indicate that CA could be used as a soil amendment to immobilize Cd. However, CA may not be an optimal amendment, because it increased residual and bioavailable Cd fractions (Table 2). Further studies are required to find soil amendments containing CA that increase only the stable Cd fraction in contaminated soils.

Feasibility of MB as an amendment for immobilization of Cd

Due to dilution, total Cd concentrations in soil decreased with increasing additions of MB (Table 4). Therefore, percentage distributions of Cd fractions in soil indicate the effects of MB on Cd bioavailability. Herein, recovery rates for Cd fractionation in incubated soils ranged from 97.4 to 103%.

Portions comprising F1 significantly decreased with increasing additions of MB, and corresponding increases were observed in F2, F3, and F5 fractions (Table 4). Specifically, F1 contents decreased by 8% following addition of MB at 40 g kg⁻¹, and F2, F3, and F5 contents increased by 3.6%, 0.7%, and 4.5%, respectively. These data indicate that bioavailable Cd fractions were transformed into non-bioavailable Cd fractions following addition of MB. Moreover, the present decreases in F1 are also likely due to CA decomposition from MB and from organic matter. As mentioned the above, a significant increases in

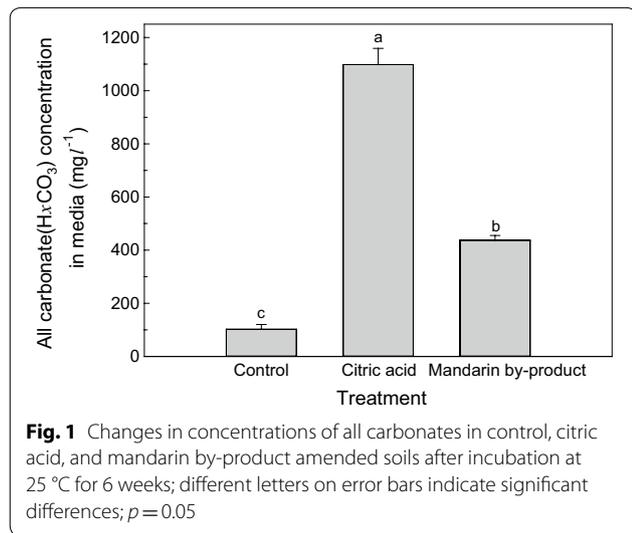


Table 3 Chemical properties of soil solutions amended with CA after a 6-week incubation at 25 °C; values with same letter within a column are significantly different; *p* = 0.05

Treatment	Soil pH (1:5 with H ₂ O)	Log activity (mol L ⁻¹)		Saturation index		
		Cd ²⁺	HCO ₃ ⁻	CdCO ₃	Cd ₃ (PO ₄) ₂	Cd(OH) ₂
Control	6.73 ^a	-5.656 ^b	-7.517 ^b	-0.435 ^b	-4.558 ^a	-4.781 ^a
CA	6.97 ^a	-5.228 ^a	-6.861 ^a	+0.572 ^a	-3.971 ^a	-4.347 ^a

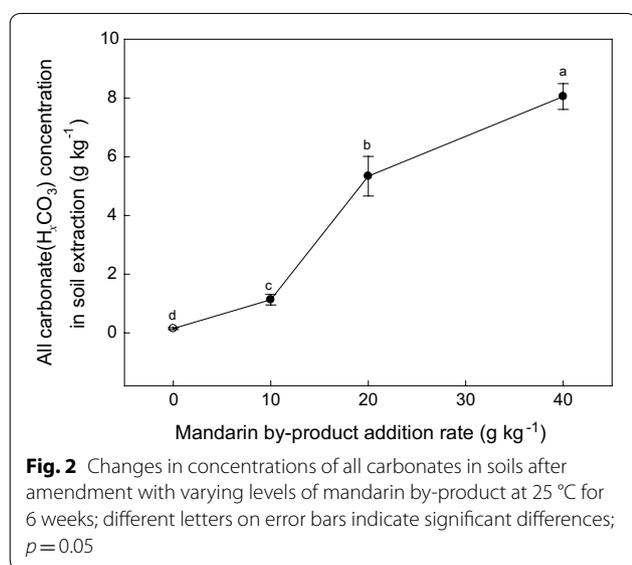
Table 4 Distributions of cadmium (Cd) fractions in soils amended with various quantities of mandarin by-product (MB) for 6 weeks at 25 °C; values with differing letters within a column are significantly different; $p = 0.05$

Addition rate of MB (g kg ⁻¹)	Cadmium fraction (%)					Cadmium concentration (mg kg ⁻¹)		
	F1	F2	F3	F4	F5	Sum ¹	Total ²	Recovery rate (%) ³
0	22.1 ^a	56.6 ^{ab}	4.5 ^a	13.2 ^a	3.6 ^c	20.9	20.4	102
10	23.0 ^a	54.9 ^b	4.6 ^a	13.5 ^a	4.0 ^c	19.7	20.0	98.5
20	18.8 ^b	57.8 ^{ab}	4.8 ^a	12.4 ^a	6.2 ^b	18.3	18.8	97.4
40	14.1 ^c	60.2 ^a	5.2 ^a	12.4 ^a	8.1 ^a	16.5	16.1	103

¹ Denotes sum of each Cd fraction concentration

² Denotes total Cd concentration measured using the aqua regia digestion method

³ Recovery rate (%) = (Sum/Total) × 100



F5 likely reflects Cd precipitation as CdCO₃ following reactions with H_xCO₃ that is generated from the decomposition of CA in MB. As shown in Fig. 2, H_xCO₃ concentrations dramatically increased with MB additions. Even though the portions of F2 and F3 did not differ significantly between treatments, the mean proportions

increased with increasing MB additions (Table 4), potentially corresponding with consequent increased negative charges of soil samples. As shown in Table 5, organic matter concentrations and negative charges of soil samples were significantly increased with greater MB contents. Moreover, Cd adsorption and complexation with organic matter increased with the application of organic matter that possess many negatively charged functional groups, such as carboxyl (COOH) and hydroxyl (OH) groups, on its external surfaces [29, 43]. The studied soil predominantly comprised kaolinite, which has a negative charge value of 4–14 cmol_c kg⁻¹. In contrast, humus reportedly has a negative charge of 130–300 cmol_c kg⁻¹ [44]. Hence, addition of the organic matter in MB dramatically increased the negative charge of soils, suggesting that portions of F2 and F3 increase with MB additions.

Soil pH dramatically decreased with additions of MB up to 20 g kg⁻¹, and thereafter increased with MB additions up to 40 g kg⁻¹ (Table 5). Various organic acids, such as ascorbic acid, lactic acid, malic acid, tartaric acid, and oxalic acid, can decrease soil pH, but organic matter with many negatively charged functional groups, such as COOH and OH, promotes increases in pH. Therefore, soil pH likely decreased due to the presence of organic acids when MB was added to up to 20 g kg⁻¹, and the

Table 5 Chemical properties of soil amended with various quantities of MB for 6 weeks at 25 °C; values with differing letters within a column are significantly different; $p = 0.05$

Addition rate (g kg ⁻¹)	Soil pH (1:5 with H ₂ O)	Total nitrogen (g kg ⁻¹)	Organic matter (g kg ⁻¹)	Negative charge of soil (cmol _c kg ⁻¹)	C/N ratio	Available phosphorus (mg kg ⁻¹)	Exchangeable cation (cmol _c kg ⁻¹)			
							Ca	K	Mg	Na
0	6.82 ^a	1.05 ^d	11.5 ^d	6.85 ^d	11.0 ^a	142 ^a	4.70 ^d	0.44 ^d	0.95 ^d	0.37 ^c
10	4.44 ^c	2.54 ^c	25.2 ^c	7.43 ^c	9.92 ^b	140 ^a	5.03 ^c	0.81 ^c	1.17 ^c	0.42 ^{bc}
20	4.55 ^c	5.18 ^b	51.5 ^b	8.70 ^b	9.94 ^b	137 ^a	5.61 ^b	1.29 ^b	1.51 ^b	0.46 ^{ab}
40	6.35 ^b	9.50 ^a	97.9 ^a	11.4 ^a	10.3 ^b	144 ^a	6.14 ^a	2.10 ^a	2.09 ^a	0.53 ^a

Table 6 Correlation coefficients for relationships between Cd fractions and chemical properties of soil after amendment with varying quantities of MB for 6 weeks at 25 °C; n = 12

Soil property	Cd fraction				
	F1	F2	F3	F4	F5
Soil pH	-0.253	+0.434	+0.141	+0.104	+0.05
Negative charge	-0.998***	+0.984***	+0.293	-0.386	+0.783**
H _x CO ₃	-0.971***	+0.537*	+0.744**	+0.623*	+0.993***

*, **, and *** denote significance at 0.01, 0.005, and 0.001 levels of probability, respectively

increase in pH following addition of 40 g kg⁻¹ MB probably reflects of the presence of organic matter. Addition of large amount of organic matter that has many negatively charged functional groups on its external surface could increase negative charge of soil and cause deprotonation from soil solution. Subsequently, this might increase soil pH. As shown in Table 5, negative charge of soil with 40 g kg⁻¹ of MB dramatically increased from that with 0, 10, and 20 g kg⁻¹ of MB. Other studies have also reported that the soil pH increased at high level of biosolid addition [29, 45].

Several studies show that the bioavailability of Cd can be limited by enhancing soil pH [23, 45–48]. However, Cd fractions were highly correlated with negative charges and concentrations of H_xCO₃ in our soil samples, rather than with soil pH (Table 6). F1 was negatively related with negative charges and H_xCO₃ concentrations in our soils. Yet F2 and F5 were positively correlated with negative charge and H_xCO₃ concentrations, further suggesting that MB immobilizes Cd by promoting adsorption and precipitation of Cd as CdCO₃ following reactions with the H_xCO₃ products of CA decomposition in MB. These reactions would be facilitated by increases in negative charges of soil surfaces that are likely induced by organic matter in MB. Unlike CA, MB increased non-bioavailable Cd fractions without increasing bioavailable Cd fractions, suggesting that as a soil amendment, MB can immobilize Cd in the soil without increasing its phytoavailability.

It will be difficult to apply MB as a soil amendment that immobilizes Cd in arable soils without concomitantly improving soil fertility and crop productivity. Although we provide no evidence of the effects of MB on crop productivity, MB improved the chemical properties of soils, as shown in Table 5. Specifically, concentrations of nitrogen, organic matter, potassium, calcium, and magnesium and cation exchange capacities of our soil samples were significantly increased with increasing additions of MB after 6-weeks incubation. However, soil pH decreased by 4.44 and 4.55 with additions of 10 and

20 g kg⁻¹ MB, respectively, and increased to 6.35 with MB at 40 g kg⁻¹. Acidic soil pH conditions near 4.44–4.55 have been shown to lower the availability of macro essential elements, such as nitrogen, phosphorous, potassium, calcium, and sulfur [49–52]. Subsequently, the low availability of these elements results in nutrient deficiencies of crops. Therefore, to prevent decreases in soil pH, one may consider combined applications of MB and lime fertilizer to arable soils. Liming materials have mainly suggested that the most effective a soil amendment to increase soil pH [32, 40, 43]. In addition, Liming materials improved not only availability of macro nutrient but also immobilization of cadmium which were attributed to the increase soil pH [32, 50]. Further studies of the combined effects of MB and lime fertilizer on Cd immobilization and soil pH are warranted.

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Authors' contributions

HHL, DYH, HRH, YLP, CR, and SUK carried out soil sampling, soil analyses, and data organization. DCS, TKO, and COH participated in interpreting the obtained results and organizing the manuscript. All authors read and approved the final manuscript.

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