



Fe-Modified Common Reed Biochar Reduced Cadmium (Cd) Mobility and Enhanced Microbial Activity in a Contaminated Calcareous Soil

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Abstract

The immobilization of soil cadmium (Cd) by biochar and modified biochar is an eco-friendly and cost-effective strategy. In the current study, the effect of raw biochar (BC) and iron-modified biochar (Fe-BC) derived from common reed on the fractionation and mobility of Cd was evaluated, as was its effect on soil microbial activity in contaminated calcareous soil. Treatments involved a combination of two factors: type of biochar (CK: Control, BC, and Fe-BC) and soil Cd concentration (0, 15, and 30 mg kg⁻¹). Treatments were applied to the soil and incubated for 90 days. The application of both biochars increased soil pH and soil organic carbon content (16.6–48.0%), microbial biomass carbon (40.5–75.1%), basal respiration (16.6–48.0%), substrate-induced respiration (12.4–41.9), and dehydrogenase activity (25.5–102.1%), while it reduced diethylene-triamine pentaacetic acid (DTPA)-extractable Cd (22.1–39.5%). The addition biochars, particularly Fe-BC, prominently decreased the concentration of exchangeable and carbonate fractions and increased the concentration of Fe-MnOx, as well as the organic and residual fractions of Cd in the soil. Moreover, relative to the control treatment, the incorporation of raw and Fe-modified biochar into 30 mg kg⁻¹ Cd-spiked soil significantly decreased the Cd mobility factor (MF) value by 14.5 and 21.8%, respectively. Fe-modified biochar had a more significant impact than raw biochar on the immobilization of Cd in the soil, and its improved soil microbial activity to a greater extent. Overall, the findings indicate that Fe-modified biochar derived from common reed can immobilize Cd and improve soil microbial attributes in contaminated calcareous soil. Therefore, it can be used as an eco-friendly amendment for restoring Cd-contaminated calcareous soil.

Keywords Cd mobility · Immobilization · Microbial attributes · Modified biochar · Sequential extraction

1 Introduction

The contamination of soils with potentially toxic elements (PTEs) has become a global concern due to its harmful effects on soil biota and plant and human health (Karimi et al. 2018; Khodaverdiloo et al. 2020). Cadmium (Cd) is a highly toxic mobile PTE that is carcinogenic to all living organisms (Karimi et al. 2018; Khodaverdiloo et al. 2020). Anthropogenic activities, such as applying agrochemicals,

smelting, mining, and irrigating wastewater irrigation, have accelerated Cd contamination in soils (Kamran et al. 2019; Rajendran et al. 2019). Cadmium is a non-biodegradable PTE and is highly persistent in soils. Cadmium has no biological role, and a high concentration of this element in soil is toxic to soil microorganisms. Toxic levels of Cd have adverse effects on microbial activities via several mechanisms, including by displacing essential nutrients, inhibiting enzyme synthesis, damaging cell membranes, and creating oxidative stress (Xu et al. 2018). Therefore, Cd-contaminated soils must be remediated if the associated environmental risks are to be restricted (Tan et al. 2020).

In situ immobilization is a known efficacious and environmentally friendly method for reducing the availability and mobility of PTEs in contaminated soil via the application of proper amendments such as biochar (Chen et al. 2020; Tu et al. 2020). Biochar is a carbon-rich material produced from pyrolysis of biomass under anaerobic or oxygen-limited conditions (IBI 2015; Wang et al. 2020). Biochar has attracted the

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attention of researchers interested in the immobilization of PTEs in contaminated soils due to its favorable sorption properties such as its microporous structure, high carbon content, active functional groups, and large specific surface areas (SSAs) (Wang and Wang 2019; Tu et al. 2020). Moreover, biochar can affect PTE availability in soil by changing specific attributes of the soil, such as pH and organic matter (OM) content. Generally, the efficacy of biochar in the immobilization of PTEs in soil depends on its physicochemical traits (Wang et al. 2020; Lyu et al. 2020; Wan et al. 2020).

The effects of biochar on soil biological attributes are often neglected in PTE-contaminated soil. Eco-friendly amendments should not only decrease the mobility of PTEs in contaminated soil, but they should also improve the soil biological status (Tang et al. 2020b). Biochar can modulate microbial activities in PTE-contaminated soils by improving the soil physical and chemical attributes, supplementing soil labile carbon and nutrients, and reducing the toxicity of PTEs in the soil (Xu et al. 2018).

Although biochar can immobilize PTEs in contaminated soil, its PTE immobilization efficiency is limited by its lack of adsorption sites. Therefore, a suitable method for improving biochar's adsorption capacity needs to be developed (Zhang et al. 2020; Wang et al. 2020; Lyu et al. 2020). Qiao et al. (2018) reported that the combined amendment of biochar and zero-valent iron (ZVI) had great potential for reducing Cd availability in contaminated paddy soil over single biochar or ZVI amendments. Chemical modifications present a promising method for improving the physicochemical traits of biochar, such as its porous structure, specific surface areas, and the surface functional groups, which can increase its capability for the stabilization of PTEs in contaminated soils (Wang et al. 2020; Zhang et al. 2020). Among the currently known chemical modification approaches, saturating the biomass with metal ions (such as FeCl_2 , MgCl_2 , and ZnCl_2) has been highlighted as an exceptional method for PTE stabilization in contaminated environments (Yu et al. 2020; Feng et al. 2020). Metal ion modification can enhance the SSA of biochar and can increase the number of oxygen-containing functional groups of biochar. These effects, in turn, enhance the interactions between the biochar and PTEs in the soil via several mechanisms, such as the formation of surface complexes, precipitation, ion exchange, and cation bonding (Li et al. 2017; Chen et al. 2020). Previous researches shows that the biomass pretreatment of FeCl_3 solutions improves the stabilization performance of PTE adsorption (Feng et al. 2018; Xu et al. 2020). Pan et al. (2019) application of Fe-modified biochar successfully decreased Cd availability in a contaminated paddy soil.

Determinations of PTEs chemical fractions in soil can be considered when investigating the immobilization performance of biochar (Boostani et al. 2019; Wang et al. 2020). It is widely confirmed that distribution of PTEs chemical fractions affects their mobility. The conversion of PTEs from

labile fractions (exchangeable and carbonate bounded) to less-labile fractions (Fe-Mn oxide bounded, organically bounded, and residual) leads to PTE immobilization (Boostani et al. 2019; Palansooriya et al. 2020; Wang et al. 2020). Previous studies have used different biochar amendments to stabilize Cd-contaminated soils (Ali et al. 2020; Xu et al. 2020). For example, Xu et al. (2020) reported that corn straw biochar reduced the availability of Cd in soil by transforming Cd into a state of lower availability. Ali et al. (2020) found that the labile fractions of Cd in smelter-contaminated soil decreased after the addition of apricot shell- and apple tree-derived biochar; meanwhile, the soil recalcitrant fractions and microbial activity increased. Qiao et al. (2019) reported that the ZVI-modified biochar decreased Cd availability in an alkaline paddy soil by enhancing the transformation of available Cd fractions into less available fractions.

Changes in Cd mobility induced by biochar can affect the microbial traits of soil (Tang et al. 2020b). However, to the best of our knowledge, there is little information available about the effect of Fe-modified biochar on the microbial attributes of contaminated calcareous soils with low OM content with varying levels of Cd. Moreover, the performance of Fe-modified common reed biochar on the stabilization of Cd in contaminated calcareous soils has not yet been studied. Thus, this study was designed to investigate the potential of raw and Fe-modified biochar to immobilize Cd and its impact on the microbial activity of contaminated calcareous soil. The results provide new insights into the development of biochar amendments for the sustainable remediation of Cd-contaminated calcareous soils.

We hypothesized that modifying common reed biochar by adding Fe would enhance its Cd stabilization capability in the soil. We also proposed that adding raw and Fe-modified biochars derived from common reed would promote the immobilization of Cd and soil microbial attributes while exhibiting a clear dependence on the biochar's traits and changes in Cd mobility and distribution.

2 Materials and Methods

The experimental soil (classified as Typic Haplocalcids) was obtained from the surface layer (0–20 cm) of an experimental farm situated in Ahvaz, Khuzestan province, SW Iran. Twenty samples from different plots were collected and mixed thoroughly to obtain a composite sample. Detailed information about the study area, soil sampling process, and analysis is given in Karimi et al. (2019). The soil sample was air-dried and sieved (< 2 mm). Afterward, specific characteristics of the soil were measured using standard laboratory methods as described in Carter and Gregorich (2008).

The raw common reed was collected, air-dried, and passed through a 2-mm sieve (Singh et al. 2017). The prepared common reed biomass was soaked in a 0.5 M FeCl₂ solution. The methods described by Singh et al. (2017) were followed when measuring the biochar's traits.

An incubation study involving nine treatments and three replications was performed following a randomized complete design in a factorial arrangement. Cadmium-contaminated soil samples were thoroughly mixed with the biochars in plastic jars. The samples were kept in an incubator for 90 days. At the end of the incubation period, all soil samples were air-dried, thoroughly mixed, and passed through a 2-mm sieve. The soil traits were measured using the same methods as those described for the initial soil. The chemical fractions of Cd in the soil were determined using the sequential extraction method of Tessier et al. (1979).

2.1 Soil Spiking with Cd

The prepared soil was spiked with salt of cadmium nitrate Cd(NO₃)₂ at concentration of 15 (Cd15) and 30 (Cd30) mg Cd kg⁻¹ dry soil. Control soil (Cd0), without addition of Cd, was also used for comparison. The soil was thoroughly mixed in plastic containers. The spiked soil was placed into plastic containers in three replicates for each treatment. The Cd-spiked soil was mixed completely and incubated at 25 °C under periodic wetting-drying moisture conditions (without drainage) for 7 months to achieve equilibrium distribution and homogenization of Cd in the soil (Karimi et al. 2017a, b; Karimi et al. 2018). After incubation period, the Cd-spiked soil was air-dried and prepared for future study.

2.2 Biochar Preparation and Characterization

The raw common reed was collected, air-dried, and passed through a 2-mm sieve (Singh et al. 2017). The prepared common reed biomass was soaked in 0.5 M FeCl₂ solution with the biomass/FeCl₂ ratio of 1:5 for 2 days then filtered and dried at 105 °C for 4 h under anoxic conditions (Feng et al. 2020). The raw and Fe-soaked common reed biomass were then pyrolyzed at 500 °C for 3 h with a heating rate of 5 °C min⁻¹ in an electrical furnace under anaerobic conditions. The N₂ flow was used to maintain anaerobic condition (Khajavi-Shojaei et al. 2020). The raw (un-modified) biochar and modified biochar were named BC and Fe-BC respectively.

The methods described by Singh et al. (2017) were used to measure biochars traits. The specific surface area (SSA) was measured by the BET (Brunauer-Emmett-Teller) method (Micromeritics Gemini 2380, US), using N₂ as the adsorbate gas. The total elemental composition (C, H, and N) of prepared biochars was

determined by elemental analyzer (Vario EL III, Germany). The scanning electron microscope (SEM) (Leo 1455 VP, UK) imaging analyses were used to evaluate the surfaces and structure of biochar samples. The surface functional groups of the biochar samples were analyzed using a Fourier-transform infrared spectrometer (FTIR) (Spectrum Perkin Elmer GX, USA).

2.3 Incubation Test and Analysis

An incubation study was performed based on randomized complete design, as a factorial arrangement, with nine treatments and three replications. Treatments were the combination of two factors: (1) soil Cd concentration at the rate of 0, 15, and 30 mg kg⁻¹ dry soil, and (2) biochar types including control treatment (CK: without the biochar addition), BC, and Fe-BC (applied at 2% w/w). Cadmium-contaminated soil samples (500 g) were thoroughly mixed with the biochars in plastic jars. Afterwards, samples were kept in an incubator for 90 days at moisture of 70% of the field water holding capacity and temperature of 25 °C.

At the end of incubation period, soil samples were air-dried, thoroughly mixed, and passed through a 2-mm sieve. The soil pH was measured in a 1:1 soil/deionized water ratio. The soil OC and available Cd concentration were measured using the same methods described above for the initial soil. Chemical fractions of Cd in the soil were determined using sequential extraction method of Tessier et al. (1979), which separated the soil Cd into five fractions: (1) exchangeable (Exch), (2) carbonates bounded (Car), (3) iron–manganese oxides bounded (Fe-MnOx), (4) organic matter bounded (OM), and (5) residual (Res) (Table SI). For evaluating the potential of biochar treatments in immobilization of Cd mobility at different levels of Cd contamination, the mobility factor (MF) of Cd in the soil was calculated as follows (Kabala and Singh 2001).

$$MF (\%) = \frac{\text{Exch} + \text{Car}}{\text{Sum of fractions}} \times 100$$

For evaluating the influence of biochar amendments on microbial activity, soil basal respiration (BR) by Anderson method (1982), substrate-induced respiration (SIR) using the method described by Alef and Nannipieri (1995), soil microbial biomass carbon (MBC) by the fumigation-extraction procedures (Jenkinson and Ladd 1981), and dehydrogenase activity (Alef and Nannipieri 1995), as important and common soil microbial indicator of soil dehydrogenase activity (DHA) is considered an indicator of overall soil microbial activity which occur intracellular in all living microbial cells (Karimi et al. 2020), were measured (Table 1).

Table 1 Some physicochemical characteristics of the experimental soil

Property	Unit	Value
Sand	g kg ⁻¹	406 ± 14
Silt	g kg ⁻¹	380 ± 12
Clay	g kg ⁻¹	214 ± 8
pH	–	7.7 ± 0.07
EC	dS m ⁻¹	2.45 ± 0.05
CEC	cmol _c kg ⁻¹	12.6 ± 0.19
Organic carbon	g kg ⁻¹	4.1 ± 0.13
CCE	g kg ⁻¹	413 ± 11
Total N	g kg ⁻¹	0.36 ± 0.02
NaHCO ₃ -extractable P	mg kg ⁻¹	13.5 ± 0.24
NH ₄ OAc-extractable K	mg kg ⁻¹	273 ± 16
DTPA-extractable Cd	mg kg ⁻¹	0.47 ± 0.09
Total Cd	mg kg ⁻¹	1.05 ± 0.15

CEC, cation exchange capacity; EC, electrical conductivity; CCE, calcium carbonate equivalent

Data are means ± standard deviation, *n* = 3

2.4 Data Analysis

Two-factor analysis of variance (ANOVA) was carried out to evaluate statistical analysis of experimental data through the SAS 9.4 software. Mean comparison of different treatment was determined by the Duncan's at *P* < 0.05.

3 Results

3.1 Characterization of the Biochars

The results showed that the physicochemical traits of biochar changed after being modified with FeCl₂ (Table 2). The Fe-BC had a higher pH, EC, total Fe concentration, and O and H content than BC (Table 2). FTIR spectroscopy was carried out to evaluate the extent to which the Fe-modification of biochar impacted the presence of surface functional groups in the Fe-BC (Fig. 1). The FTIR spectroscopy results showed that the intensities of bands of oxygen-containing functional groups in Fe-BC treatment were significantly higher than in the BC treatment (Fig. 1).

3.2 Soil pH and SOC

The soil pH increased after the application of BC (0.30–0.33 unit) and Fe-BC (0.16–0.23 unit) for all Cd concentration levels (Fig. 2). Significant differences in soil pH were observed when the BC and Fe-BC treatments were compared. However, no significant differences in soil pH were found

Table 2 Some physicochemical characteristics of the biochars used in this study

Property	Unit	Biochar	Fe-modified biochar
pH	–	10.18 ± 0.07	10.34 ± 0.06
EC	dS m ⁻¹	6.8 ± 0.06	7.1 ± 0.08
CEC	cmol _c kg ⁻¹	23.5 ± 0.3	67.4 ± 0.4
SSA	m ² g ⁻¹	68.8 ± 0.2	132.2 ± 0.3
Biochar recovery	%	29.3 ± 0.9	34.2 ± 1.1
Ash	%	33.6 ± 0.5	30.7 ± 0.4
Fixed C	%	4.56 ± 0.24	8.37 ± 0.38
C	%	61.1 ± 1.34	49.3 ± 1.04
O	%	3.04 ± 0.28	17.6 ± 0.3
H	%	1.06 ± 0.08	1.19 ± 0.12
N	%	0.88 ± 0.04	0.82 ± 0.02
C/N	–	80.91 ± 1.36	70.48 ± 1.16
Total P	g kg ⁻¹	1.18 ± 0.04	1.19 ± 0.04
Total K	g kg ⁻¹	2.78 ± 0.22	2.79 ± 0.16
Total Fe	mg kg ⁻¹	375.7 ± 12.6	501.9 ± 14.5
Total Cd	mg kg ⁻¹	BD	BD

EC, electrical conductivity; CEC, cation exchange capacity; SSA, specific surface areas; C, carbon; O, oxygen; N, nitrogen; H, hydrogen; Cd, cadmium; BD, below detection

Data are means ± standard deviation, *n* = 3

when the Cd0 and other Cd concentrations (Cd15 and Cd30) were compared.

Relative to the control condition, the soil SOC content increased proportionally with the application of BC (3.29–3.3 fold) and Fe-BC (2.93–2.99 fold) for all soil Cd concentrations (Fig. 2). Again, no significant differences in soil SOC content were observed when comparing the different soil Cd concentration levels (Cd0, Cd15, and Cd30).

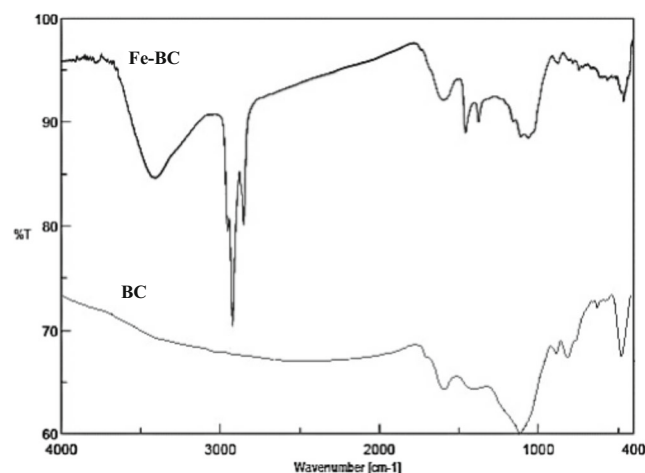


Fig. 1 The Fourier-transform infrared (FTIR) spectra of the biochars. BC, raw biochar; Fe-BC, Fe-modified biochar

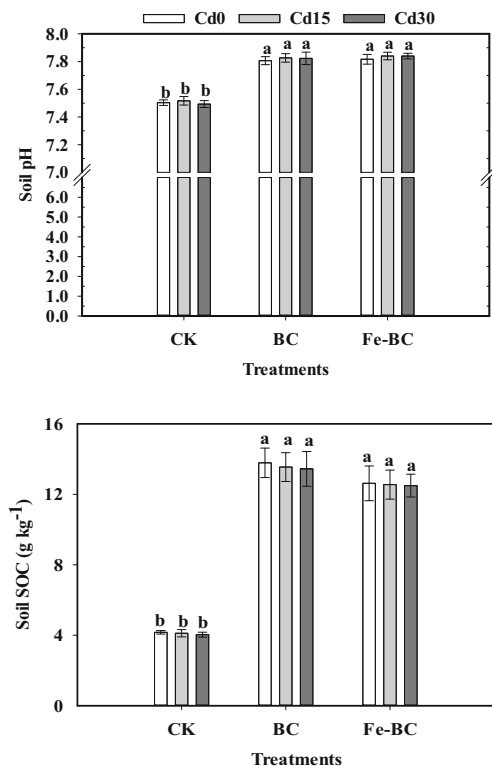


Fig. 2 pH and soil organic carbon (SOC) in different treatments. Different letters show the significant difference according to Duncan's test at 5% probability level ($n = 3$). CK, control; BC, raw biochar; Fe-BC, Fe-modified biochar

3.3 Availability and Fractionation of Cd in Soil

The results indicated that for different Cd levels, adding BC and Fe-BC to the soil significantly decreased the availability of Cd (DTPA-extractable Cd) in the soil by 22.1–28.6% and 33.1–39.5%, respectively, when compared to the control treatment (Fig. 3). Moreover, the soil exchangeable Cd fraction significantly decreased at the Cd15 and Cd30 levels after the addition of BC (by 11.3 and 21.5%), and Fe-BC (by 25.1 and 24.6%) when compared with the control treatment (Fig. 3).

The addition of BC and Fe-BC biochars decreased the carbonate Cd fraction in the soil when compared to the control at all Cd levels (Fig. 3). At all Cd concentration levels, the application of Fe-BC reduced the carbonate Cd fraction more substantially than the application of BC in the soil. The concentration of Cd bound to Fe-MnOx in the soil increased after the addition of BC (7.3–14.3%) and Fe-BC (11.3–21.5%) at all Cd levels (Fig. 3). Furthermore, when compared to the control treatment, the application of both biochar types significantly enhanced the binding of Cd to OM (2.3- to 5.5-fold) and residual (1.3–2.15-fold) fractions at all Cd levels (Fig. 3).

The relative percentage of Cd in control soil at all Cd levels in the soil was as follows: Car > Fe-MnOx > Exch > Res > OM (36.5–46.1% and 2.1–4.8% for Car and OM fraction, respectively); whereas in the Fe-BC-amended soil at Cd15 and Cd30, the

trend was as follows, respectively: Car > Fe-MnOx > Res > OM > Exch, and Car > Fe-MnOx > Res > Exch > OM (Fig. 4). Also, in BC treatment, Cd fractions at Cd15 and Cd30 were in the following order, respectively: Car > Fe-MnOx > Res > Exch > OM, and Car > Fe-MnOx > Exch > Res > OM. In all treatments, up to 35% of the total Cd in the soil was in Car fraction, and 20.7% in Fe-MnOx Cd fraction (Fig. 4).

Overall, the results of the proportion of Cd fractions at different levels of Cd in the soil clearly demonstrated that the application of BC and Fe-BC treatments considerably changed the Cd distribution in the soil and caused the Cd to convert from the Exch fraction to the OM and residual fractions (Fig. 4), thus reducing Cd bioavailability. The results also indicated that Fe-BC had a greater effect on decreasing the proportion of Cd Exch and Car fractions than that of BC.

3.4 Cd Mobility in Soil

The mean comparison of the mobility factor (MF) values at different Cd concentrations in the soil (Fig. 5) demonstrated that this factor significantly decreased with the addition of BC (10.8–11.4%) and Fe-BC (11.4–14.5%) compared to the control treatment (Fig. 5). In addition, the Cd mobility increased with increasing the Cd levels from Cd0 (44.2–56.8) to Cd30 (56.1–66.1) in the soil (Fig. 5). There were no significant differences in the biochar impact on the changes in Cd mobility factor at various levels of Cd in the soil (Fig. 5), which revealed that both BC and Fe-BC treatments had high efficiency in the Cd immobilization at different concentrations of Cd in the soil.

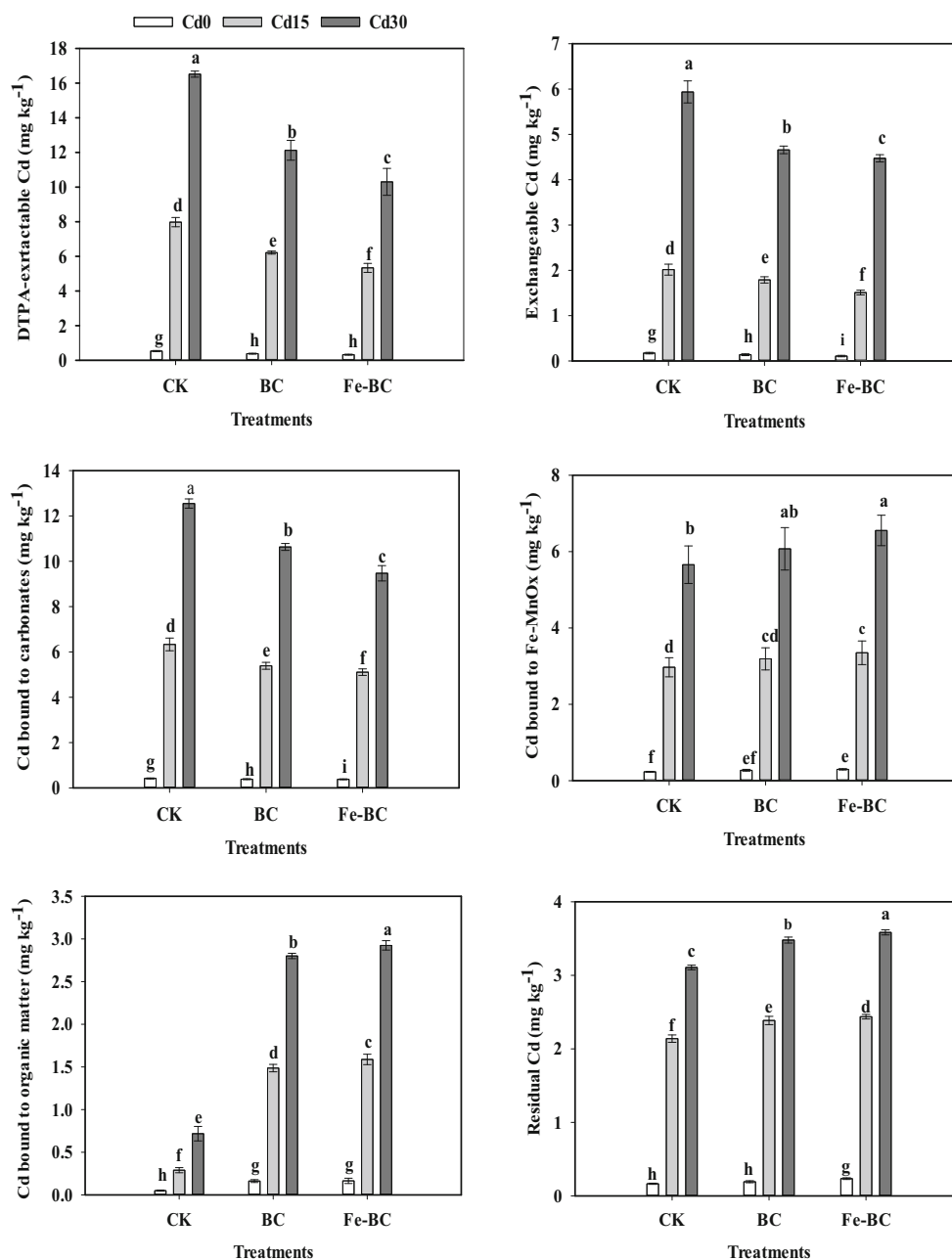
3.5 Soil Microbial Attributes

The results indicated that with increasing the Cd concentration in the soil, the soil MBC, BR, and SIR values significantly decreased (Fig. 6). The increases in Cd levels in the soil also led to a decrease in soil dehydrogenase activity (Fig. 6). At all Cd levels in the soil, the BC and Fe-BC addition significantly increased the soil MBC (40.5–75.1%), BR (16.6–48.0%), SIR (12.5–41.9%), and dehydrogenase activity (25.5–102.2%) values compared to those of the control treatment.

4 Discussion

The results of the biochar traits (Table 2) indicated that Fe was successfully loaded onto the biochar. This result is supported by the SEM image of the biochars, which showed that the Fe particles were more prominent in the Fe-BC than in the BC (Fig. S1). Overall, the results regarding the physicochemical traits and FTIR spectra

Fig. 3 DTPA-extractable Cd and Cd distribution in exchangeable (Exch), bound to carbonates (Car), bound to iron–manganese oxides (Fe-MnOx), bound to organic matter (OM), and residual fractions (Res) in different treatments. Different letters show the significant difference according to Duncan's test at 5% probability level ($n = 3$). CK, control; BC, raw biochar; Fe-BC, Fe-modified biochar



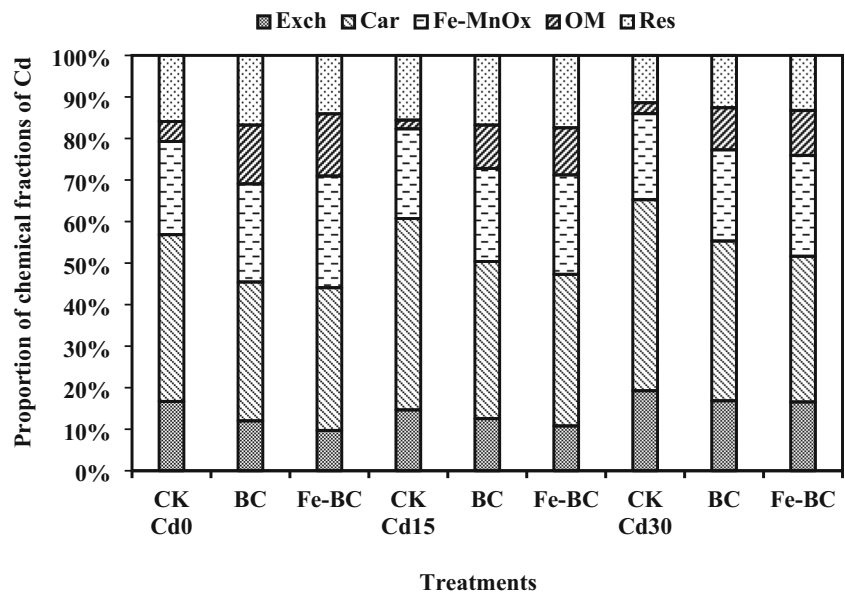
of the biochars suggest that Fe-BC has a better adsorption capability than BC and perhaps more sorption sites for Cd stabilization.

Soil pH was most strongly affected by biochar alkalinity (Karimi et al. 2019; Tang et al. 2020a). The results showed that the biochar applied to the soil in this study has a high content of alkaline ions, such as potassium (K) (Table 2), which can neutralize the hydrogen ions in soil. Therefore, the increase in soil pH that accompanied the biochar treatments (BC and Fe-BC) could be attributed to the alkaline nature of these biochars. Moreover, the liming effect of the biochars could increase the soil CEC and decrease the amount

of H⁺ (Tang et al. 2020a), which could further explain the increase in soil pH after the addition of biochars.

In other work, Karimi et al. (2020) also reported that a significant increase in the pH of calcareous soil that was amended with corn-residue biochar. The biochars used in the current study had a high carbon content (Table 2). Therefore, the application of the biochars used in this study caused a significant increase in soil SOC content. Our results agreed with the findings of Karimi et al. (2020), who reported that the addition of corn biochar increased the SOC content of calcareous soil under incubation conditions. Saffari et al. (2020) also reported that the SOC content in a sandy loam soil

Fig. 4 Proportion of Cd fractions in different biochar treatments. Exch, exchangeable; Car, bound to carbonates; Fe-MnOx, bound to iron–manganese oxides; OM, bound to organic matter; Res, residual; CK, control; BC, raw biochar; Fe-BC, Fe-modified biochar



significantly increased due to the incorporation of corn-residue biochar.

The decrease in the DTPA-extractable Cd observed in the present study after the addition of biochars could be attributed to changes in Cd fractions in the soil. Soil pH strongly influences the reactions and availability of PTEs in calcareous soil (Karimi et al. 2019). In comparison to the control treatment, treatments that involved the addition of biochars to soil increased the soil pH in the current study (Fig. 2). The increased soil pH values accelerated the formation of sorption sites on the negatively charged soil colloids and clay particles (Xu et al. 2020). In addition, the increased soil pH due to the application of biochars can result in the formation of Cd hydroxide (Cd(OH)₂) or phosphate precipitation, which can

reduce the availability of Cd and promote its transition to low labile fractions (Zhao et al. 2016).

Therefore, the increase in soil pH resulting from biochar addition can be responsible for the decrease in soil Cd availability. This conclusion is confirmed by the negative linear regressions observed between the soil pH and DTPA-extractable Cd ($R^2 > 0.83$, $P < 0.01$, Fig. S2). Congruent with our results, Tu et al. (2020) found that the application of maize biochar led to a significant decrease in the DTPA-extractable Cd in alkaline soil. The biochar application can enhance the exchangeable content of alkaline cations such as K, Ca, and Mg, which might promote the adsorption of Cd through the cation exchange and, in turn, reduce the concentration of exchangeable Cd in the soil (Xu et al. 2020). The results revealed that the Fe-BC reduced the Cd availability in the soil to a greater extent than BC. This result was probably due to the higher Cd stabilization of the Fe-BC treatment, which promotes adsorption (either by ionic exchange or by complexation), electrostatic attraction, and precipitation due to its unique physicochemical properties (Table 2).

The results showed that the application of both biochars decreased the carbonate Cd fraction in the soil (Fig. 3). This result may be due to the adsorption of Cd bound to carbonate by biochars through different mechanisms like complexation, precipitation, and physicochemical sorption (Chen et al. 2020). Furthermore, Cd bound to carbonate fraction was probably transformed to Cd bound to OM fraction. Therefore, the Cd carbonate fractions declined when BC and Fe-BC were added to the soil. The applied biochars, particularly Fe-BC, had abundant oxygen-containing functional groups on their surfaces (Fig. 1), which can react with the organic-bound Cd, thus increasing Fe-MnOx fractions. Moreover, the increase in soil pH transforms Cd from other fractions into the Fe-MnOx fraction following the formation of precipitate

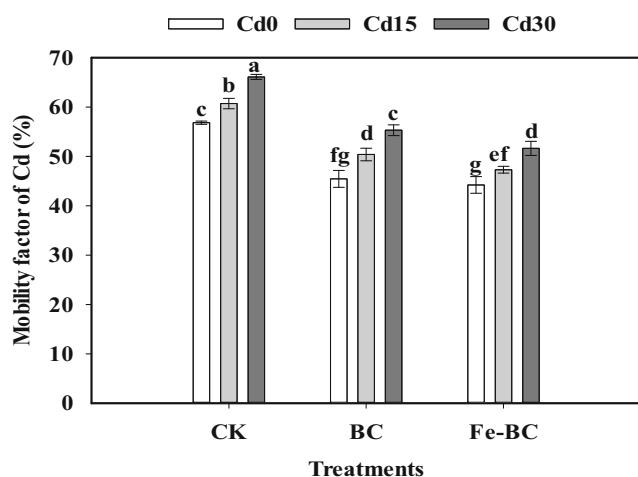
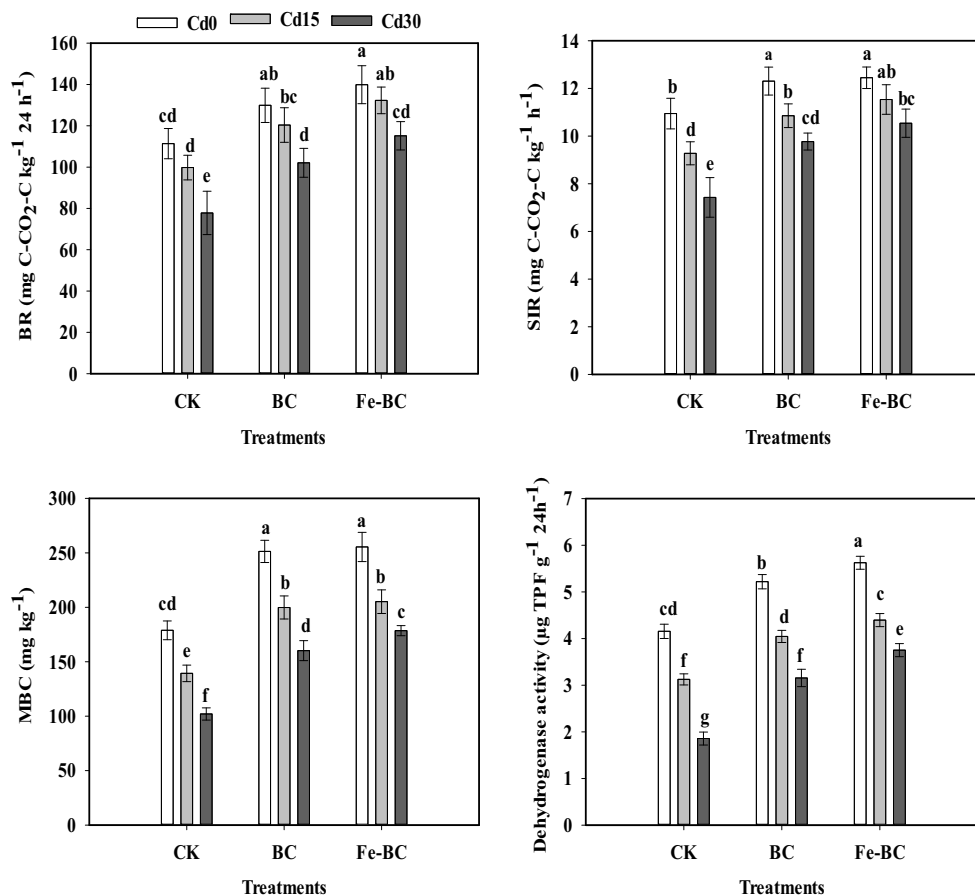


Fig. 5 Cd mobility factor in different biochar treatments. Different letters show the significant difference according to Duncan’s test at 5% probability level ($n=3$). CK, control; BC, raw biochar; Fe-BC, Fe-modified biochar

Fig. 6 Soil basal respiration (BR), substrate-induced respiration (SIR), microbial biomass carbon (MBC), and dehydrogenase (DEH) activity in different treatments. Different letters show the significant difference according to Duncan's test at 5% probability level ($n = 3$). CK, control; BC, raw biochar; Fe-BC, Fe-modified biochar



complexes and increased negative charges, which, in turn, decreases Cd mobility (Chen et al. 2020). This finding is in line with previous observations of other PTE-contaminated calcareous soils amended with different biochars (Hamzenejad Taghliabad and Sepehr 2018; Boostani et al. 2019).

The increased OM-Cd fractions associated with biochar treatments was probably due to the complexation between the elevated SOM with BC and Fe-BC addition and mobile metal ions in the soil. The results also indicate that the OM-Cd fraction was higher in the Fe-BC treatment than in the BC treatment in Cd-contaminated soil, which may be due to the greater oxygen-containing functional groups of the former (Fig. 1). Perhaps Cd showed a high preference for the carboxyl and hydroxyl functional groups of biochar surface (Tu et al. 2020). Therefore, the increased binding of Cd to OM fraction in this study might generate more oxygen-containing functional groups, mineral oxides, and OM of biochars. Ahmad et al. (2014) reported that the high immobilization of PTEs in biochar-amended soils was mainly due to the presence of mineral oxides and OM in biochar, which coprecipitated and made inner-sphere complexes with PTEs. Similar to the current study, other studies indicated that biochar concentration of PTEs

bounded to OM in calcareous soil increased with the addition of different biochars (Boostani et al. 2019; Kabiri et al. 2019; Karimi et al. 2019).

In terms of the MF, which is a relative dynamic index of Cd, among the chemical fractions of Cd, the Exch and Car fractions are identified as mobile and having a high environmental risk (Kabala and Singh 2001). A high MF value is indicative of high PTE bioavailability (Kabala and Singh 2001; Boostani et al. 2019). The most significant relative reduction in MF value (14.4%) was observed with the application of Fe-BC by for the Cd30 treatment (Fig. 5). The results demonstrated that the applied biochars (especially Fe-BC) significantly immobilized the Cd in the soil. General mechanisms for soil PTE immobilization with the addition of biochars were as follows: (1) the enhanced soil pH (Fig. 2) and the introduction of ligands, which can reduce the Cd mobility via the formation of insoluble precipitate, which is supported by the strong negative relationship between the MF of Cd and soil pH ($R^2 > 0.88$, $P < 0.01$) (Fig. S3), and (2) the increased soil OM (Fig. 2) that can bind to Cd and increase the adsorption and complexity of Cd through the activated surfaces of functional groups and, thus, the formation of stable complexes (Lahori et al. 2017).

In addition, mobile Cd cations transformed into low labile fractions (Fig. 4), perhaps via the formation of Cd

(hydro-)oxide phosphate or carbonate. The formation of these compounds could be due to the existence of these anions, as shown in a previous study by Zhang et al. (2016). The lower values of MF associated with the Fe-BC treatment than BC demonstrated that Fe-BC had a substantial impact on Cd immobilization, which could be explained by some of its characteristics (e.g., higher pH, CEC, and specific surface area (Table 2), and its surface functional groups (Fig S2). These factors could have reduced Cd mobility via adsorption and the formation of surface complexes.

The presence of Fe in the Fe-BC treatment (Table 2) might have decreased Cd mobility via ion exchange mobility. Decreased PTEs mobility was also reported by Xu et al. (2016) in Cd- and Pb-contaminated soil that was treated with rice and wheat straw. Nonetheless, further research is needed to understand the interaction between Cd and biochars in the pedosphere in the long term. Ahmad et al. (2017) reported that a surface complexation including Fe-/Al-minerals or carboxyl groups and PTEs-phosphate precipitation significantly reduce PTE mobility in alkaline soil amended with biochars. Boostani et al. (2018) also reported that the MF of soil Cd in contaminated calcareous soil considerably decreased due to the addition of various types of biochars.

The decreases in MBC, BR, and SIR values due to Cd contamination (Fig. 6) could be attributed to increased Cd availability (Fig. 3) and mobility (Fig. 6) and possible Cd toxicity. This is confirmed by the significant negative correlation between soil Cd availability and soil microbial attributes (Table 3). Similarly, Xu et al. (2018) noticed that the microbial biomass values in Pb- and Cd-spiked soils were significantly lower than in non-spiked soil. In addition, the decrease in soil dehydrogenase activity (Fig. 6) associated with increased soil Cd levels could be due to Cd ions reactions with enzyme-substrates or their interactions with protein active groups (Tang et al. 2020b).

High concentrations of Cd in soil have a toxic effect on almost all microbes by affecting metabolic functions such as

protein synthesis (Tang et al. 2019; Pan et al. 2020), thus causing variations in microbial biomass and activity (Kaurin et al. 2018). Although the total contents of Cd can exert either positive or negative impacts on microbial activity (Fajardo et al. 2019; Pan et al. 2020), in the present research, Cd fractions were dominant drivers of changes in the microbial activity of soil.

In the current study, the Cd level affected the microbial attributes of soil. However, the effects of its fractions, especially its mobile fractions (Exch and Car), were of particular interest since they might be more toxic to soil microorganisms than other fractions. The present findings also demonstrated that the Fe-MnOx fraction of Cd significantly impacts the microbial activity of soil. Pearson's correlation coefficients (r) between chemical fractions of Cd and soil microbial attributes demonstrated that, among the five fractions of Cd in the soil, the Exch, Car, and Fe-MnOx fractions had the strongest effects on soil microbial attributes. These fractions were also toxic for soil microorganisms and inhibited microbial activity (Table 3). These effects can be attributed to the high mobility and bioavailability of these fractions in soil. In contrast, no significant correlations were noted between OM and the residual fractions of Cd and soil microbial attributes (Table 3).

The biochar amendments applied in the present study had significant positive impacts on all investigated soil microbial attributes (Fig. 6). Increases in the soil microbial biomass and respiration owing to the addition of biochar can be explained by the following factors: (1) increased soil SOC (Fig. 2) and supplemented labile carbon and soluble nutrients for microbial utilization (Xu et al. 2018; Herrmann et al. 2019); (2) reduced soil Cd availability (Fig. 3) and mobility (Fig. 5) and the subsequent decrease in Cd toxicity; (3) increased substrate availability and the release of accessible organic compounds (Karimi et al. 2020); and (4) the provision of suitable microbial habitat (Xu et al. 2018; Karimi et al. 2020). Biochar also acts as a source of energy for soil microbes and facilitates their survival in contaminated soils (Ali et al. 2020).

Table 3 Pearson's correlation coefficients (r) between different chemical fractions of Cd and soil microbial attributes ($n = 27$)

Soil variable	DTPA-extractable Cd	Cd MF	Exch	Car	Fe-MnOx	OM	Res
MBC	0.90**	-0.82**	-0.76**	-0.66**	-0.52**	-0.23 ^{ns}	-0.36 ^{ns}
BR	0.84**	-0.74**	-0.63**	-0.61**	-0.46*	-0.18 ^{ns}	-0.32 ^{ns}
SIR	0.91**	-0.77**	-0.68**	-0.63**	-0.49*	-0.20 ^{ns}	-0.35 ^{ns}
DEH	0.89**	-0.89**	-0.83**	-0.73**	-0.59**	-0.29 ^{ns}	-0.38 ^{ns}

**Significant at 1% probability level

*Significant at 5% probability level

^{ns} Not significant

Cd MF, Cd mobility factor; *Exch*, exchangeable Cd; *Car*, Cd bound to carbonates; *Fe-MnOx*, Cd bound to iron-manganese oxides; *OM*, Cd bound to organic matter; *Res*, residual Cd; *MBC*, microbial biomass carbon; *BR*, basal respiration; *SIR*, substrate-induced respiration; *DEH*, dehydrogenase activity

Similar results were reported for PTE-contaminated soils amended with various biochars (Xu et al. 2018; Tang et al. 2020b). Dehydrogenase is an important intracellular enzyme that is associated with soil microbial activity (Karimi et al. 2020; Tang et al. 2020b). The enhanced dehydrogenase activity associated with biochar addition can be explained by the resultant increases in soil SOC (Fig. 2) and microbial biomass (Fig. 6). In addition, the higher dehydrogenase values of the Fe-BC treatment (in comparison to the BC treatment) might be due to the greater microbial biomass of the Fe-BC treatment (Fig. 6). This difference was confirmed by the significant correlation between dehydrogenase activity and MBC values ($R = 0.95$, $P < 0.05$; Table S2).

In general, the soil SOC in the BC treatment was higher than that in the Fe-BC treatment. Nevertheless, the soil microbial biomass values of the Fe-BC-treated soil were higher than those of the BC-treated soil for all soil Cd levels, most notably at the Cd30 level. These results could be attributed to the higher SSA of the Fe-BC soil, the presence of Fe (Table 2), and the lower Cd availability, mobility, and toxicity associated with the Fe-BC soil. These results are supported by the strong negative correlations found among Cd availability, Cd mobility, and soil microbial attributes (Table 3). Moreover, the results of the current study revealed that the improvements in the microbial attributes of Cd-contaminated soil were due not only to the OC supplied by the biochar but also to its Cd immobilization capability.

5 Conclusions

In the current study, raw and FeCl₂-modified common reed biochars were applied to Cd-contaminated calcareous soil. These biochar efficacies to immobilize Cd in soils with different Cd concentrations were evaluated for both types of biochar. Associated improvements in the soil microbial attributes were also assessed. The results showed that the Cd concentration did not affect biochars efficiencies to immobilize Cd. Both the applied biochars, particularly the FeCl₂-modified biochar, increased the alteration of potential mobile fractions (exchangeable and carbonate) of Cd to organic and residual fractions. This effect was found to reduce Cd's mobility and enhance the soil microbial activity. Soil pH and OM significantly changed the Cd distribution of the tested soils. Among the different chemical fractions of Cd in the soil, exchangeable Cd had the most significant correlation with variations in soil microbial attributes. The FeCl₂-modified biochar outperformed the raw biochar in terms of Cd immobilization and soil microbial attribute improvements. Cadmium availability was found to be the main factor that regulated microbial activity. Based on the results of the present work, FeCl₂-modified common reed biochar can be used as a soil remediation material to improve soil microbial activity and minimize

Cd mobility in soil. However, further long-term studies are needed to explore the interactions among Fe-modified biochars, soil microorganisms, and Cd in contaminated calcareous soils.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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