



# Impact assessment of Zeolite, Ca-bentonite and Biochar amendments on Cd bioavailability and fractions in polluted calcareous soils

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## Abstract

The refining of polluted soils by heavy elements is one of the most important environmental policies in industrialized and developing countries. Using adsorbents is a suitable procedure for the immobilization of heavy metals in polluted soils. This study aimed to assess the immobilization of Cadmium (Cd) in polluted calcareous soil affected by the application of organic and inorganic amendments including Biochar (from grape pruning residues) and natural Zeolite and their interaction under wheat cultivation. The treatments used in this study were two amendments of Zeolite and Biochar (from grape pruning wastes) at three levels (0, 1, and 4%) and three levels of Cd contamination (0, 75, and 150 mg/kg soil). A 16-week incubation period was considered for the homogenization of the amendments in soil and wheat was grown according to the standards procedure. At the end of incubation, different fractions of Cd including residual, exchangeable, bonded to organic matter, bonded to carbonate and bonded to iron and manganese. Also available Cd by DTPA and EDTA methods and 1000-grain weight of wheat were measured. The results showed that the highest amount of Cd bound to organic matter was obtained in 4% Biochar treatment to 15 mg/kg. The highest and lowest amounts of Cd extracted with DTPA were obtained in the control one (92 mg/kg) and the level of 4% Biochar (67 mg/kg), respectively. The results showed that increasing the amount of Biochar and Zeolite amendments increased the weight of 1000 grains of wheat in all treatments. According to the results of the study, the use of Biochar and Zeolite reduced the amount of Cd extracted by DTPA (82.436 mg/kg) and EDTA (115.605 mg/kg). Finally, the results showed that the use of Biochar and Zeolite has reduced active Cd and its mobility in the soil due to increasing organic and carbonate fractions. Combining biochar and zeolite in soil remediation efforts can enhance their effectiveness in reducing the concentration and mobility of active Cd. The biochar provides a stable carbon matrix for long-term immobilization of Cd, while the zeolite offers additional adsorption capacity and ion-exchange capabilities. This synergistic effect can lead to improved soil quality and reduced environmental risks associated with Cd contamination.

**Keywords** Wheat · Toxicity · ETDA · Heavy metals · Immobilization

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## Introduction

Soil pollution from heavy metals, notably Cadmium (Cd), is a pressing global issue, with around 10% of the world's soils currently contaminated (Kinuthia et al. 2020; Rahimi et al. 2021). These contaminants, introduced into the food chain through soil accumulation and plant absorption, pose significant health risks to living organisms due to their rapid bioaccumulation and long half-life (Yue et al. 2017). Human activities such as metal extraction, wastewater discharge, and agricultural practices are the primary sources of heavy metal pollution in soils (Raicevic et al. 2009; Yari et al. 2017; Rahimi et al. 2021). Unlike organic pollutants, heavy metals persist in soil, inhibiting enzymatic activities, damaging protein structures, and impeding plant growth (Vig 2003). Cd recognized as a human carcinogen, poses severe health risks, including kidney, bone, and nervous system damage, with a permissible daily intake limit of 70 µg/day (Waalkes 2000; Gallicchio et al. 2021). Cd's low binding tendency in soil enhances its absorption by plants, leading to toxicity in plant leaves and disrupting crucial processes like seed germination, photosynthesis, and enzyme activity (Kabata-Pendias and Pendias 1992; Wang et al. 2015). Consequently, high Cd concentrations in the food chain pose significant dangers to humans, causing kidney and liver diseases, bone problems, and neurological disorders (Kabata-Pendias 2010; Nishijo et al. 2017; Balali-Mood et al. 2021). Efforts to mitigate heavy metal pollution are essential to preserve food security and safeguard human health.

The escalating soil contamination by heavy metals has spurred extensive research into soil remediation methods to safeguard both environmental and human health (Bhattacharyya and Gupta 2008; Kim et al. 2022). Various remediation techniques have been proposed, including the utilization of zeolites and bentonite (Mathialagan and Viraraghavan 2002; Dodangeh et al. 2018). Zeolites, hydrated aluminosilicates with porous structures, exhibit reversible dehydration and cation exchange properties, making them effective adsorbents for heavy metal removal (Mahabadi et al. 2007; Mahesh et al. 2018; Cataldo et al. 2021). Studies suggest that zeolites promote the formation of oxides, carbonate-metal deposits, and complexes, thereby reducing the solubility of heavy metals (Shi et al. 2009; Hamidpour et al. 2010). Bentonite, a common aluminosilicate clay mineral, has been utilized as geochemical barriers in nuclear waste repositories and hazardous chemicals landfills (Garcia-sanchez et al. 1999). These materials offer promising avenues for mitigating heavy metal contamination in soils, thus contributing to the preservation of environmental quality and human well-being.

The most significant and primary clay mineral is montmorillonite, which possesses all the characteristics of

bentonite when influenced by it. Bentonite has been recognized as a highly effective substance for absorbing heavy metals in polluted soils due to its exceptional physico-chemical properties, including its extensive surface area, high cation exchange capacity, strong affinity for adsorbing both organic and inorganic pollutants, low permeability, cost-effectiveness, widespread availability in most soils (Alabarse et al. 2011). The acceptable structure of montmorillonite, the most crucial component of bentonite, was described by (Marshall 1935) According to these findings, montmorillonite is composed of two thin layers of silica tetrahedra with a thin layer of alumina octahedra in between (Bhattacharyya and Gupta 2008). Previous research has shown that bentonite is a suitable material for concentrating and solidifying heavy metal ions from large volumes of aqueous solutions (Garcia-sanchez et al. 1999).

Biochar, derived from plant biomass and agricultural waste, emerges as a cost-effective and readily available solution for heavy metal removal and soil improvement (Yue et al. 2017; Dai et al. 2018). Its porous structure and surface functional groups facilitate heavy metal adsorption and immobilization, thereby reducing their toxicity and enhancing soil quality (Huang and Yuan 2016). Research indicates that biochar application can decrease the mobility and bioavailability of heavy metals like Cd, while also increasing soil pH to support plant adaptation and growth (Penido et al. 2019). Various studies demonstrate biochar's effectiveness in immobilizing heavy metals such as Cd in soil. (Janoš et al. 2010; Lahori et al. 2020; Zhu et al. 2020; Yang et al. 2021). Additionally, biochar derived from rice straw has been found to reduce Cd bioavailability compared to controls (Zhang et al. 2017b). Understanding the chemical forms and interactions of heavy metals within soil components is crucial for assessing environmental risks accurately (Bogusz and Oleszczuk 2018). The employment of sequential extraction techniques proves valuable in ascertaining the chemical states of metals within the soil (Bogusz and Oleszczuk 2018). Sequential extraction techniques help identify metal fractions in soil, with exchangeable forms being more accessible and active, while residual forms are less reactive (Prusty et al. 1994). By leveraging biochar and understanding heavy metal chemistry in soils, effective strategies can be devised for mitigating environmental contamination and promoting ecosystem health.

Investigating the impact of Zeolite, Ca-bentonite, and Biochar amendments on the bioavailability and distribution of cadmium (Cd) in contaminated calcareous soils is mandatory for addressing environmental and health concerns. This research aims to help ensure food safety, promote sustainable agriculture, and contribute to the advancement of scientific knowledge in the field of soil remediation and contamination management. The risk of Cd pollution in the

soil is not a function of the total amount of this element, and its fractions play a very important role in the toxicity of this element and the use of various amendments should be based on these fractions. Also, the remediation of polluted soil should be based on environmentally friendly materials so that these materials do not cause pollution in the next step. Research on the remediation of Cd contamination in calcareous soils using zeolite and biochar, as well as the exploration of their impact on Cd fractions, is limited. Therefore, it is imperative to delve into these fractions through further investigation. DTPA (diethylenetriaminepentaacetic acid) and EDTA (ethylenediaminetetraacetic acid) are chelating agents commonly used to extract heavy metals from soil. Chelating agents like DTPA and EDTA form stable complexes with heavy metal ions, facilitating their release from soil particles. DTPA is often preferred for extracting heavy metals like Zn, Cu, Fe, Mn, and Co, while EDTA is more effective for extracting metals such as Pb, Cd, and Ni. These chelating agents work by surrounding the heavy metal ions, forming water-soluble complexes that are easily extracted from the soil matrix. This process mimics the natural mechanisms by which plants absorb heavy metals from the soil, making DTPA and EDTA extractions relevant for assessing the bioavailability of heavy metals to plants. Overall, DTPA and EDTA extractions provide valuable information about the potential risk of heavy metal contamination to plants and can aid in soil remediation efforts and environmental monitoring. Since the effect of different amendments on different forms of Cd is not yet known, so the main aims of this study include: (1) assess the impact of three amendments including Zeolite Ca-bentonite and Biochar at two levels on changes of different fractions of Cd, and (2) comparison of DTPA and EDTA extractors affected by two types of amendments and (3) the effect of different levels of pollution and amendments on some properties of wheat.

## Materials and methods

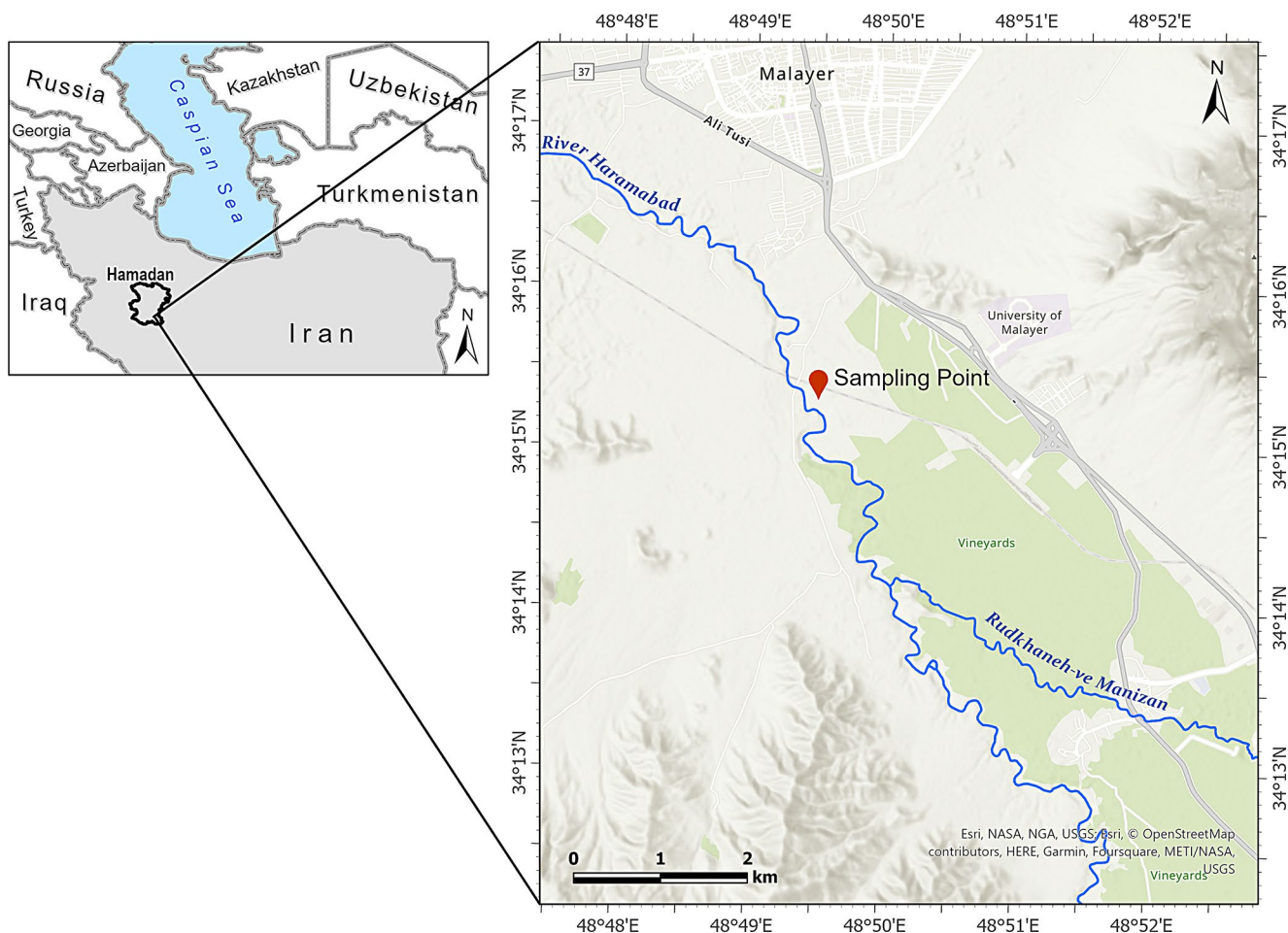
### Soil sampling and soil properties

This research was conducted in Agriculture Research Center in Malayer city, Hamadan province located in west of Iran (Fig. 1). For this study, a soil sampling was conducted at 48°49' 30" E; 34° 15' 33" N from a wheat farm. Soil samples were collected in labeled polyethylene bags from topsoil (0–30 cm) and transferred to the laboratory. Initially, approximately 1 kg of the specimen underwent air-drying, followed by disintegration in a porcelain mortar and subsequent sieving with a 2 mm mesh. Soil pH was assessed in a suspension of a 1:5 soil-to-water ratio using an ohmmeter 744 pH meter, following the methodology outlined by

Thomas in 1996. The electrical conductivity (EC) of the soil samples was determined using the Rhoades method (1996). Organic matter (OM) content was ascertained through wet oxidation, as per the procedure outlined by Walkley and Black in 1934, while Cation Exchangeable Capacity (CEC) was determined employing the Bower procedure detailed by Rowell in 1994. Particle size distribution (PSD) was derived utilizing the hydrometer method, following the protocol established by Bouyoucos in 1962. The equivalent  $\text{CaCO}_3$  was quantified via titration, adhering to the method proposed by Sims in 1996.

### Treatment

To investigate the effect of different modification on bioavailability and distribution of Cd chemical forms, the experiment was conducted in a completely randomized design with 3 replications. Zeolite and Bentonite samples were synthesized from Khoro Biyabank and Anarak mines located in Isfahan province, respectively. The raw materials underwent a process of pulverization and subsequent air-drying at 25 degrees Celsius, followed by sieving through a 2 mm mesh to achieve the desired particle size for rigorous scientific analysis. Biochar was derived from grape wood and was produced by collecting samples, air-drying, and crushing them into small pieces. Moisture removal was conducted in an oven at 105 °C. To establish anaerobic conditions, the prepared samples were placed in an iron cylinder (dimensions: 7 cm by 31 cm) and converted into Biochar using an electric furnace at 500 °C, with a controlled temperature increase of 3 °C per minute for 2 h (Cantrell et al. 2012). The resulting amendments, including Zeolite, Bentonite, and Biochar, were transferred to a laboratory, where a comprehensive analysis of some of their chemical properties was performed. Cd nitrate was employed to contaminate the soil at three pollution levels of Cd (0, 75, and 150 mg/kg soil), and the contaminated samples were incubated at 25 °C for 16 weeks. Different incubation times have been considered in different studies such as Liao et al. (2005) (10 d, 20 d, 40 d, and 60 d); Rafati-Rahimzadeh et al. (2017), (30 years) Zhang et al. (2017a) (12 months). Moisture content was maintained at approximately 70% of the field capacity using distilled water. After the incubation period, the samples were air-dried and sieved through a 2 mm mesh. The soil and modifier of the soil were then placed in plastic containers at three concentration levels (0, 1, and 4%), followed by wheat cultivation in the soils. After 32 weeks, Cd bioavailability was assessed using DTPA and EDTA extractants, and total Cd content was determined through a digestion method (1:3  $\text{HNO}_3/\text{HCl}$ ) (Sungur et al. 2014; Capelli et al. 2014; Bankaji et al. 2023). Additionally, the weight of 1000 grains of wheat was measured at the end



**Fig. 1** The location of the sampling point

**Table 1** Summarizes the sequential extraction method

Chemical forms of Cd	Extractant	Temperature (°C)	Shaking time (h)	pH
Exchangeable	Mg(NO <sub>3</sub> ) <sub>2</sub> , 1 M	25	1	7
Bonded to Carbonate	Na OAc, 1 M	25	5	5
Oxide Fe-Mn- bound	NH <sub>2</sub> OH-HCL, 0.04 M	96	6	2
Bonded to OM	NaOH	85	3	2
Residual	HNO <sub>3</sub> A	120	3	-

of the experiment. Some chemical properties of modifiers were measured as follows: pH and electrical conductivity (EC) were determined using a 1:5 ratio of modifier to water with a pH meter and an EC meter, respectively. Organic carbon was measured by oxidation with potassium dichromate (Chapman 1965). The distribution of Cd in soil samples was analyzed using the Tessier et al. (1979) method, which involved measuring five soluble and exchangeable components, organic, carbonate, oxide Fe-Mn bound, and residual fractions (Table 1). The concentration of Cd in the extracts

was determined using Atomic Absorption Spectrometry (AAS) techniques (Varian 220). Fractionation and the impact of EDTA and DTPA extractants were assessed at the concentration of 150 mg Cd/kg soil during the cultivation of wheat at three levels (0, 75, and 150 mg/kg soil).

### Statistical analysis

Statistical analysis for this study was conducted using SAS 9.2 software. Means were compared utilizing a one-way ANOVA test by Duncan's test at a probability level of 5%. Graphs were generated using Excel 2016 software providing the visual representation of the data.

## Results and discussion

### Soil properties

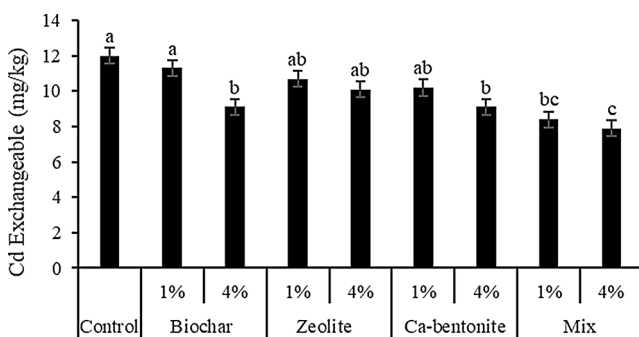
The physical and chemical properties of the soil under investigation are detailed in Table 2. The soil exhibits a

**Table 2** The physical and chemical properties of the examined soil

Parameter	Value	Unite
Sand	30	(%)
Silt	46	(%)
Clay	24	(%)
Textural class (USDA)	Silty loam	-
pH	7.724	-
EC	0.443	(dS/m)
CaCO <sub>3</sub>	17.902	(%)
OM	1.661	(%)
CEC	23.011	(meq/100gr soil)
Available Cd	0.205	mg/kg soil
Total Cd	2.223	mg/kg soil

**Table 3** Chemical properties of Zeolite and Biochar used in the experiment

Treatment	pH	EC (dS/m)	CEC (meq/100gr soil)	OC (%)	Total Cd (mg/kg)
Biochar	9.631	0.242	34.000	71.000	0.000
Zeolite	8.324	0.862	33.407	0.000	nd
Ca-bentonite	8.235	0.102	51.116	0.000	0.010



**Fig. 2** Impact of different treatments on exchangeable Cd

moderately silty loam texture, accompanied by an organic matter content of 1.661% and an equivalent CaCO<sub>3</sub> content of 17.902%. The pH of the soil, a pivotal factor influencing heavy metal availability, is determined to be 7.724. Notably, this elevated pH has the potential to diminish the accessibility of heavy metals, particularly Cd, in the soil. The research of Khodaverdiloo and Samadi (2011) emphasized the significance of activated calcium carbonate in facilitating the absorption and retention of Cd in calcareous soils. It is crucial to acknowledge that higher soil pH can induce the precipitation of heavy metals into various forms such as hydroxides, phosphates, or carbonates as highlighted by Kabata-Pendias in 2000.

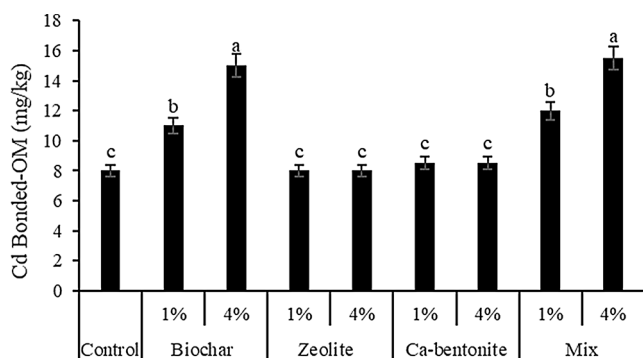
The findings indicate a substantial presence of carbon within the Biochar as outlined in Table 3. This notable content of organic carbon (71%) not only addresses a deficiency of organic matter in agricultural soils but also exerts beneficial influences on the chemical and physical properties of the soil. The pH levels of the employed Biochar and Zeolite

were recorded at 9.631 and 8.324, respectively, suggesting their potential to mitigate the accessibility of heavy metals, such as Cd, in accordance with previous research (Khodaverdiloo and Samadi 2011).

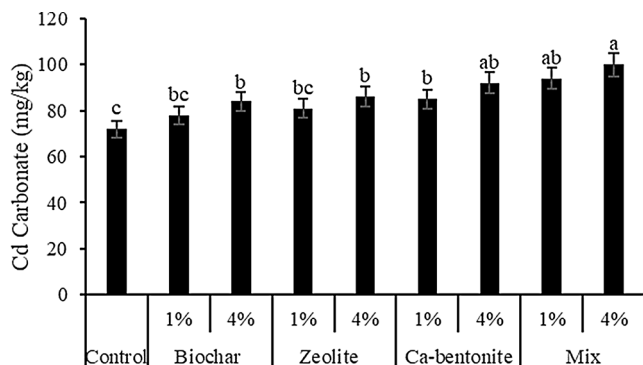
### Effect of Biochar, Ca-bentonite, and Zeolite on the distribution of cd forms

#### Exchangeable cd

Figure 2 illustrates the impact of various treatments on the quantity of exchangeable Cd in the soil. The results demonstrate that the application of Biochar, Zeolite, and Ca-bentonite treatments has effectively reduced the amount of exchangeable Cd in the soil. As presented in Fig. 2, no statistically significant difference is evident in the amounts of exchangeable Cd between various levels of Zeolite and the control group. In Biochar treatments at the 4% concentration, there was a noteworthy reduction (24%) in the levels of exchangeable Cd compared to the control group, while at the 1% Biochar concentration, the decrease in exchangeable Cd was not statistically significant. Consistent with studies conducted in Iran, Mahabadi et al. (2007b) demonstrated that the application of Zeolite in contaminated soils led to a diminished concentration of available Cd for plant uptake. The presence of additional Zeolite in the soil raised the pH, thereby reducing the competition of hydrogen ions with metal elements for adsorption by ligands and fostering the formation of more stable forms of elements. Furthermore, the increased cation exchange capacity of the soil resulting from Zeolite application enhances the exchange of metals within the Zeolite structure (Peng et al. 2009). Similarly, in a year-long incubation experiment involving different levels of rice straw and bamboo bio-charcoal added to contaminated soil, Yang et al. (2016) reported a significant decrease in the soluble and exchangeable forms of copper, aligning with the findings of this study regarding Cd. Bashir et al. (2018) also demonstrated that both Biochar and Zeolite (Mix) contribute to a reduction in the levels of exchangeable Cd, corroborating the outcomes of this investigation. Zhao et al. (2016) found that the addition of Ca-bentonite decreases the levels of exchangeable Cd. Additionally, Sprynskyy et al. (2007) proposed that increasing the mineralization of Zeolite with sewage sludge reduces the concentration of the mobile form of Cd. In a study evaluating the impact of Zeolite on reducing the availability of Cd in polluted soil in the Guilan province of Iran, Mahabadi et al. (2007b) concluded that the use of Zeolite effectively decreased the accessibility of Cd in the soil.



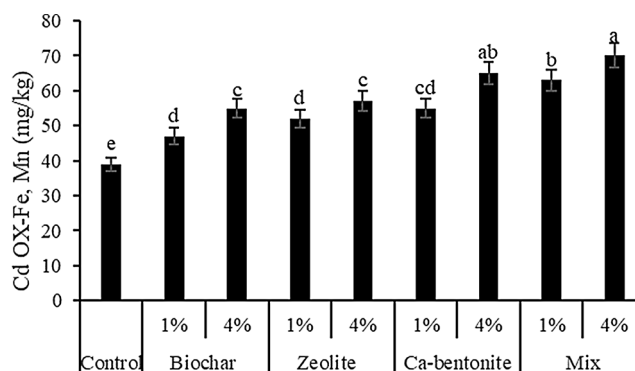
**Fig. 3** The effect of different treatments on Cd bonded to organic matter



**Fig. 4** The impact of various treatments on Cd bonded to carbonate

### Cd bonded to organic matter

Figure 3 delineates the outcomes of different Biochar and Zeolite treatments on Cd binding to organic matter. Evidently, the incorporation of Biochar into the soil resulted in a significant increase in the concentration of Cd bound to organic matter. The most pronounced increase in Cd binding to organic matter occurred with the application of 4% Biochar (15 mg/kg, equal to 87%). Comparing the combination treatment of Biochar and Zeolite (Mix) at a 4% level with the same treatment at a 1% level, a notable increase in Cd binding to organic matter was observed. Specifically, there was a 29% increase compared to the lower concentration and a substantial 93% increase compared to the control treatment. In contrast, the inclusion of Zeolite did not induce any discernible alteration in the Cd content associated with organic matter, maintaining a comparable level to the control group (8 mg/kg), as reported by Zhang et al. (2017b). Their investigation revealed that Biochar elevated the oxidative and organic fractions of Cd while significantly diminishing the exchangeable fraction. Furthermore, Shirvani et al. (2006) substantiated that soils characterized by elevated organic matter exhibited an ability to form complexes with Cd, thereby mitigating its toxicity. It is noteworthy that although organic compounds can diminish the availability of heavy metals such as Cd by increasing the cation



**Fig. 5** The impact of various treatments on Cd bonded to iron and manganese oxides

exchange capacity of the soil, their prolonged decomposition may occasionally result in re-release, thereby increasing their availability. Thus, the utilization of non-degradable materials like Zeolite may potentially prevent the re-release of these metals into the soil. The findings of this study suggest that the simultaneous application of Biochar and Zeolite, along with the augmentation of plant nutrients, holds promise for reducing Cd availability in the soil.

### Cd bonded to carbonate

Figure 4 illustrates the impact of different amendments on alterations in Cd binding to carbonate. The results indicate a notable increase in Cd bound to carbonate with the addition of both amendments. The highest concentration of Cd carbonate was obtained in the 4% Zeolite treatment (86 mg/kg). Additionally, it has been demonstrated that increasing the Biochar from 1 to 4% leads to a rise in the content of Cd bonded to carbonate. Zeolite, identified as a porous alkaline aluminosilicate mineral with a negative charge and a high cation exchange capacity (Mohamed 2001), is increasingly employed as a soil amendment, especially for the immobilization of heavy metals. Some studies (Ostroski et al. 2009) suggest that Zeolite induces the formation of oxidative and carbonate forms of heavy elements in the soil, thereby reducing the solubility of these metals and decreasing their absorption and concentration in plants. Biochar, owing to its elevated adsorption potential resulting from structural modifications during pyrolysis of organic material, can diminish the availability of heavy metals in the soil (Uchimiya et al. 2010).

### Cd bonded to iron oxide and manganese

The outcomes of the impact of varied amendment levels on the concentration of Cd associated with iron and manganese oxides are delineated in Fig. 5. Notably, the incorporation of Zeolite and Biochar has resulted in a substantial increase in

Cd content within the fraction associated with iron and manganese oxides. Specifically, the inclusion of Biochar ranging from 1 to 4% led to a significant increase in Cd within this fraction, reaching up to 15%. In a comparable study, Shen et al. (2016) introduced Biochar (derived from broadleaf wood prepared at 600 °C for 16 h) into contaminated soil in a field experiment. Their findings indicated a significant reduction in the soluble + exchangeable, carbonate, and organic forms of zinc, accompanied by a notable rise in the fraction of zinc bonded to iron oxides and residuals. The authors proposed that the increase in pH and competitive absorption might have triggered the redistribution of zinc in the soil following Biochar addition. The introduction of Biochar was found to prompt heavy metals to preferentially bond in more stable forms, such as complexes with organic matter. This competitive effect was associated with a decrease in exchangeable zinc and carbonate, a phenomenon previously documented by other researchers (Ahmad et al. 2014). The elevation of soil pH resulting from Biochar application can be attributed to the dissolution of hydroxides and carbonates present in Biochar (Lucchini et al., 2014). Cumulatively, effective mechanisms such as ion exchange, electrostatic interaction, settling, and the formation of surface complexes have been identified as pivotal processes contributing to the immobilization of heavy metals in soils treated with Biochar (Wang et al. 2018).

### Residual cd

The results detailing the impact of Biochar and Zeolite treatments on the residual quantity of Cd are presented in Fig. 6. The findings demonstrate a significant increase in residual Cd levels with the introduction of varying concentrations of Biochar and Zeolite. Specifically, the minimum and maximum recorded residual Cd amount in the control treatment was 8.335 mg/kg and 13.546 mg/kg, respectively. Interestingly, the inclusion of Zeolite ranging from 1 to 4% did not exhibit a substantial variance in residual Cd. In contrast, elevating the Biochar concentration from 1 to 4% resulted in a notable 23% escalation in residual Cd. Previous research by Burlakovs et al. (2012) reported a significant decrease in the bioavailability of copper in contaminated soil upon the addition of Zeolite, suggesting its potential as a soil amendment for polluted environments. In agreement, Hamid et al. (2020) observed a reduction in Cd content in the soil with the utilization of Biochar. Shi et al. (2009) asserted that Zeolites play a role in transforming available forms of Cd into non-absorbable forms, thereby mitigating Cd uptake by plants. The high specific surface area, substantial cation exchangeable capacity, and the ability to form exchangeable complexes, providing adsorption capacity for ions of various sizes, are widely acknowledged by researchers as

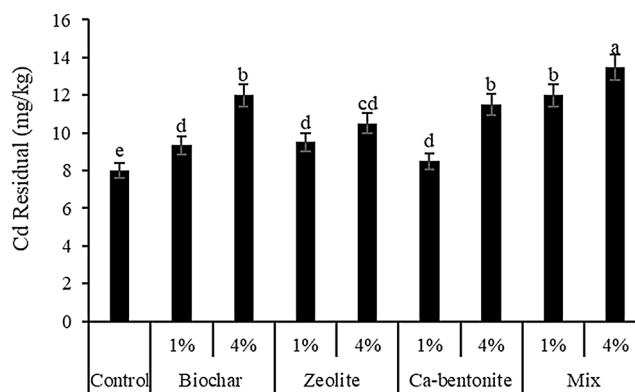


Fig. 6 Effect of different treatments on residual Cd

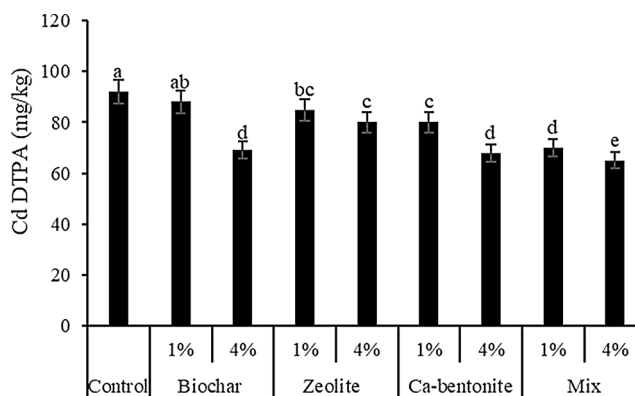


Fig. 7 The influence of different treatments on Cd extracted with DTPA

indicative of the exchange capacity of Zeolites (Babel and Kurniawan 2003). Additionally, Wen et al. (2016) noted a decrease in the exchangeable and organic forms of zinc, accompanied by a significant increase in the residual form, in a zinc-polluted sedimentary soil treated with Zeolite, corroborating the findings of the present study.

### DTPA extracted cd

Figure 7 illustrates the influence of different amendment levels on the quantity of Cd extracted through DTPA. Notably, the control treatment exhibited the highest DTPA-extracted Cd amount at 92 mg/kg, while the 4% Biochar level showed the lowest at 67 mg/kg. The results collectively suggest that the incorporation of Biochar and Zeolite has led to a reduction in Cd extraction by DTPA. This phenomenon can be attributed to the role of Zeolite in enhancing soil absorption sites, thereby diminishing the extractable Cd with DTPA. Furthermore, it is observed that the introduction of Biochar and Zeolite at the two to four% level has resulted in a decrease in Cd extracted by DTPA. Specifically, the addition of 4% Biochar demonstrated a 30% reduction in DTPA-extracted Cd. This decrease can be ascribed to various factors, including the highly porous

structure, surfactants, high dispersion, specific surface area, and cation exchange capacity of Biochar. Biochar effectively immobilizes heavy metals through mechanisms such as adsorption, ion exchange, surface complexation, and deposition, as documented by Bian et al. (2014). Peng et al. (2009) also highlighted that increasing soil dispersion and cation exchange capacity through the addition of Zeolite can immobilize and mitigate the extractability of heavy metals. Similarly, Cui et al. (2016) employed Biochar to immobilize Cd in soil, observing the highest removal of Cd in the presence of Biochar. They identified factors such as pore structure, functional groups, high carbon content, and elevated pH as contributors to the reduction of Cd bioavailability. The processes of immobilization and adsorption of heavy metals in soils treated with Biochar involve diverse mechanisms, including ion exchange, chemical adsorption, complexation, and surface interactions, as outlined by Usman et al. (2016).

### Extracted cd by DTPA and EDTA

#### Extracted cd

Figure 8 presents the results of a comparative analysis of the mean effects of various treatments on Cd extracted by EDTA. The findings reveal a substantial decrease in the quantity of Cd extracted by EDTA upon the application of Biochar and Zeolite. In the control treatment, the highest Cd extraction by EDTA was recorded at 131 mg/kg, while the lowest was observed in the 4% Zeolite treatment (96 mg/kg). The increase in the dosage of both amendments resulted in a reduction in Cd extracted by EDTA. Although the decrease in Cd extraction was not statistically significant in the Biochar amendment, a noteworthy and statistically significant decline in Cd extraction was evident in the case of Zeolite. In a parallel study, Sadegh et al. (2012) utilized 30 g/kg of pistachio compost in calcareous soils with varying textures for 60 days, observing a substantial reduction in the amount of copper extracted by EDTA compared to the control treatment. The researchers hypothesized that the decomposition of this organic matter likely generated high molecular weight organic acids, forming stable and robust complexes with copper, thereby diminishing the extractability of copper from the soil. Corroborating these results, Zhang et al. (2017) demonstrated that the incorporation of mineral amendments such as Zeolite led to a decrease in the concentration of Cd extracted by DTPA and EDTA. Furthermore, Sun et al. (2016) elucidated that compounds such as clay minerals, characterized by a high cation exchange capacity, can mitigate the dissolution and mobility of heavy metals through adsorption and deposition processes.

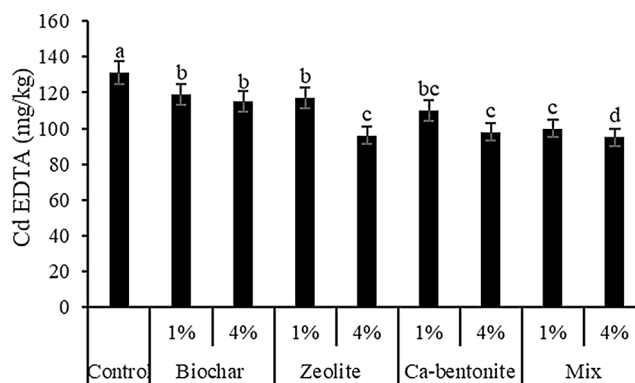


Fig. 8 The impact of different treatments on Cd extracted with EDTA

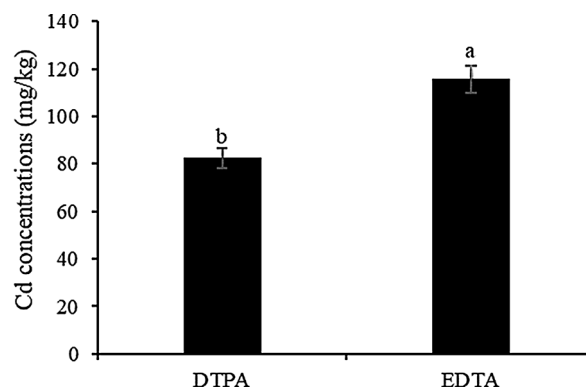


Fig. 9 Comparison of mean Cd extract with EDTA and DTPA

### Comparison of DTPA and EDTA extractants

Figure 9 illustrates the outcomes of Cd concentration using two distinct extractants. A notable difference is observed between the two extraction methods, with the EDTA extraction exhibiting higher Cd concentration compared to DTPA. This disparity can be attributed to soil pH, a critical factor governing the availability of heavy metals in the soil. With the recorded soil pH of 7.72, the alkaline nature of the soil has the potential to reduce the availability of heavy metals, including Cd, as noted by Khodaverdiloo and Samadi (2011). Activated calcium carbonate, a significant property in calcareous soils, plays a crucial role in the uptake and storage of Cd, with high soil pH leading to the deposition of heavy metals in forms such as hydroxides, phosphates, or carbonates (Kabata-Pendias 2000). Several studies have demonstrated that the incorporation of Biochar and Zeolite increases soil pH (Medyńska-Juraszek et al. 2020; Burahevskaya et al. 2021). As soil pH rises, the Cd extraction capacity of EDTA also increases. Karak et al. (2016) further support this observation, indicating that EDTA exhibits higher efficiency than DTPA in soils with a pH exceeding 7, while DTPA performs better in acidic pH conditions.

### Effect of different modifiers on weight of 1000 grains

Figures 10 and 11, and 12 depict the outcomes of a comparative assessment of the average influence of Zeolite, Ca-bentonite, and Biochar on the weight of 1000 grains of wheat. These graphical representations illuminate a discernible decrease in the weight of 1000 grains with the escalation of Cd pollution levels. However, the application of these soil amendments has elevated the weight of 1000 grains, thereby mitigating the toxicity of Cd to a certain extent. The most substantial improvement in the weight of 1000 grains occurred in treatments with 4% Zeolite, Ca-bentonite, and Biochar in uncontaminated Cd soil, registering weights of 55.66 g, 55.30 g, and 61.00 g, respectively. In the absence of Cd pollution (Cd0), the utilization of these amendments resulted in an increased wheat grain yield due to improvements in the physical and chemical attributes of the soil. In instances of Cd pollution, these amendments function as a barrier, impeding the absorption of Cd by the plant and reducing its soil toxicity. The augmentation of Zeolite, Ca-bentonite, and Biochar from 0 to 4% in the case of 150 mg/kg Cd led to a 30%, 21%, and 22% increase in the weight of 1000 grains of wheat, respectively. The application of Zeolite, Ca-bentonite, and Biochar significantly reduced the concentration of Cd in wheat grain. This reduction is primarily due to Zeolite’s ability to bind Cd ions in the soil, thereby decreasing their availability for plant uptake. Similarly, Ca-bentonite and Biochar also demonstrated strong adsorption properties, effectively immobilizing Cd in the soil and preventing its absorption by wheat roots. Zeolite, Ca-bentonite, and Biochar improved soil properties by enhancing nutrient retention and water-holding capacity. These improvements create a more favorable environment for wheat growth, indirectly contributing to healthier plants that are better equipped to cope with heavy metal stress. Healthier plants with robust root systems are less likely to

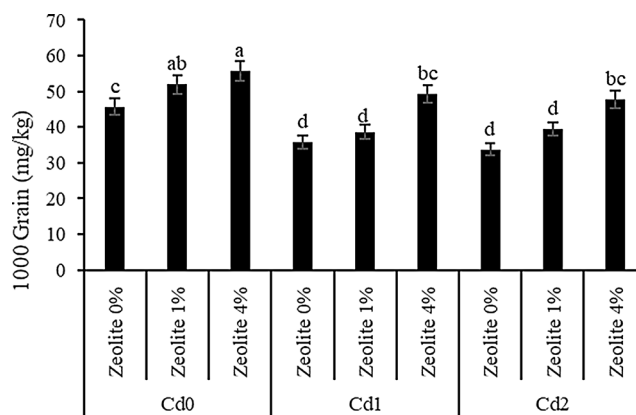


Fig. 10 The impact of Zeolite on the weight of 1000 grains of wheat

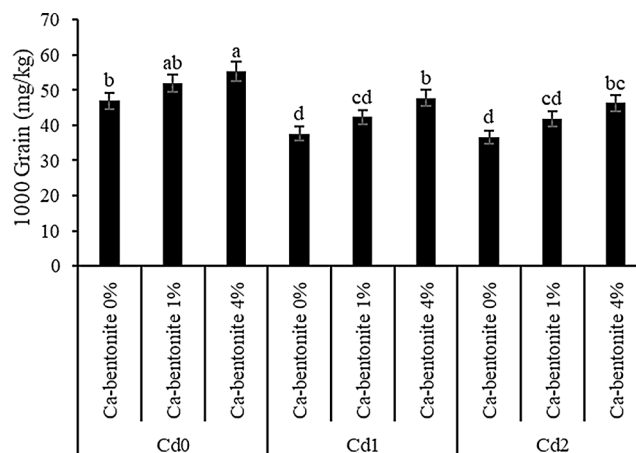
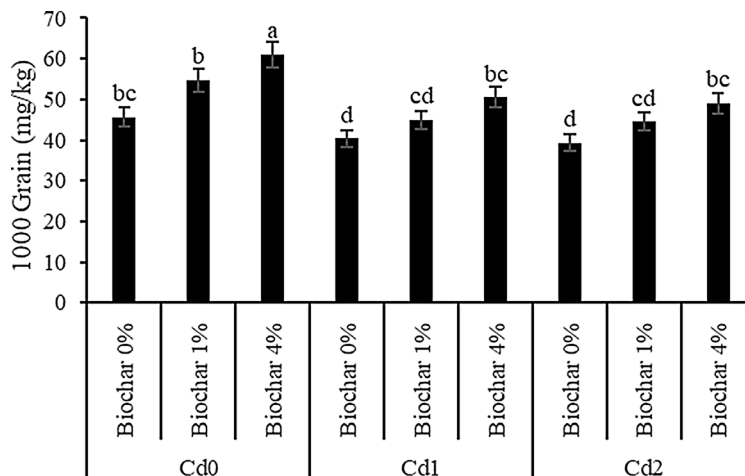
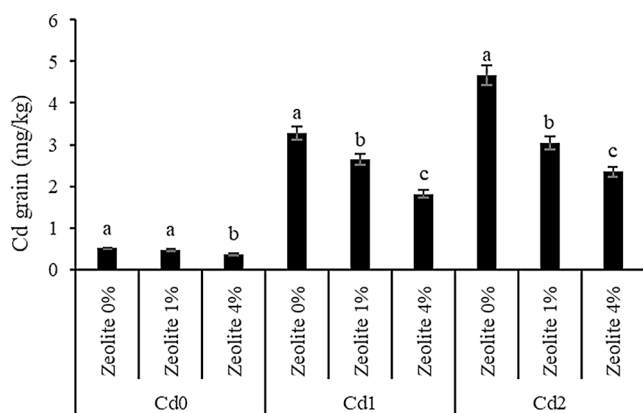


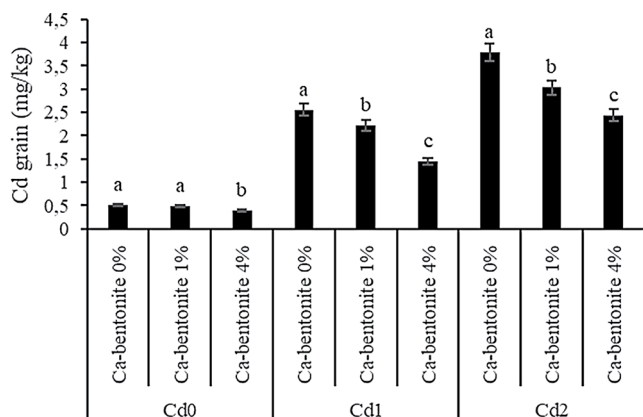
Fig. 11 The impact of Ca-bentonite Biochar on the weight of 1000 grains of wheat

Fig. 12 The impact of Biochar on the weight of 1000 grains of wheat





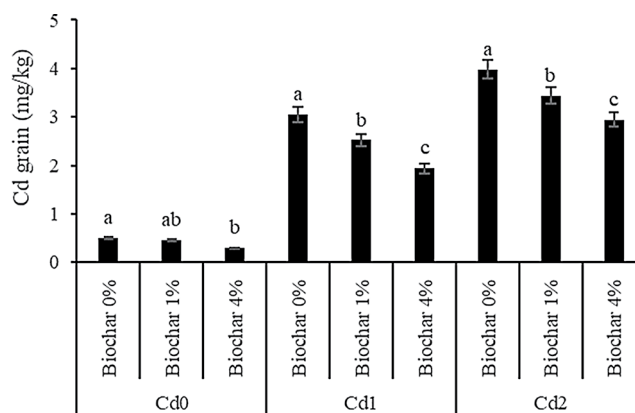
**Fig. 13** The effect of Zeolite on the grain Cd



**Fig. 14** The effect of Ca-bentonite on the grain Cd

correct amount of these amendments is crucial, as excessive use could potentially alter soil pH or disrupt the availability of other essential nutrients. The mechanisms behind the reduction in Cd uptake are attributed to the ability of Zeolite, Ca-bentonite, and Biochar to adsorb Cd and other heavy metals. These soil amendments immobilize Cd in the soil matrix, preventing its uptake by wheat roots. Zeolite's high cation exchange capacity, Ca-bentonite's expansive surface area, and Biochar's porous structure all contribute to their effectiveness in binding Cd and reducing its bioavailability.

This enhancement in the cation exchange capacity of the soil, facilitated by the concurrent application of Zeolite, has the potential to reduce the availability of Cd for plant uptake. Thus, in turn, diminishes the risk of Cd absorption by the plant through an increase in the soil's absorption sites, as documented by Wang et al. (2009) and Mahar et al. (2015). Biochar, with its distinctive physicochemical structure, contributes to improved soil fertility and crop yield, especially in degraded soils, by enhancing soil quality and health. Chaudhry et al. (2016) emphasize its positive impact on soil properties and crop yield. Gebremedhin and Haileselassie (2015) observed increased wheat yield in biochar-treated plots, attributing it to improved water and nutrient



**Fig. 15** The effect of Biochar on the grain Cd

retention, thereby enhancing plant nutrition. Abbas et al. (2017) corroborated these findings in their assessment of biochar's influence on wheat growth, highlighting its capacity to improve soil properties, alleviate Cd toxicity, raise pH levels, increase water retention, enhance nutrient availability, and ultimately stimulate plant growth and yield.

### Effect of different modifiers on grain cd

Figures 13 and 14, and 15 present the results of the impact of Zeolite, Ca-bentonite, and Biochar on the Cd content in wheat grains. These figures illustrate a significant increase in Cd concentration in grains with escalating Cd pollution. However, the application of the amendment has led to a reduction in Cd content, effectively decreasing the toxicity of Cd to some extent. The highest Cd concentration in grains was observed in the treatment with 0% Zeolite, Ca-bentonite, and Biochar in polluted soil (150 mg/kg Cd), measuring 4.66 g, 3.78 g, and 3.98 g, respectively. In Cd-polluted treatments, the amendments act as barriers, preventing the absorption of Cd by the plants and thereby reducing its toxicity in the soil. The augmentation of Zeolite, Ca-bentonite, and Biochar from 0 to 4% in the case of 150 mg/kg Cd resulted in a 50%, 36%, and 26% decrease in Cd content in wheat grains, respectively. Zeolite, Ca-bentonite, and Biochar exhibit the capability to reduce Cd bioavailability in the soil by adsorbing and immobilizing Cd ions, limiting its uptake by wheat plants. Zeolite, in particular, enhances cation exchange capacity (CEC) and improves nutrient retention in the soil. Ca-bentonite increases calcium levels, potentially competing with Cd for uptake by plant roots. Biochar, by modifying soil pH, organic matter content, and nutrient availability, indirectly influences Cd uptake by wheat, contingent on the type and dosage of Biochar. The availability of Cd for plant uptake can be diminished by amendments that transfer it from more labile fractions (such as exchangeable fractions) to less labile fractions. Supporting this, Sun et al. (2015) observed

a reduction in the quantity of Cd in *Oryza sativa L.* roots and shoots with the addition of bentonite to the soil. Zhang et al. (2017) found a significant decrease in Cd concentration in lettuce shoots grown on soils with low levels of potentially toxic element (PTE) pollution, attributed to the application of Ca-bentonite derived from rice straw. In a recent study, Bashir et al. (2018) modified Zeolite and Biochar produced from rice straw in acidic Cd-contaminated soil, leading to a substantial decrease in the amount of Cd absorbed by water spinach.

## Conclusion

This investigation explores the impact of Biochar derived from grape pruning residues, natural Zeolite, and Ca-bentonite on Cd immobilization in a Cd-contaminated calcareous soil. The results demonstrate a noteworthy reduction in the quantity of exchangeable Cd in the soil following treatments with Zeolite, Ca-bentonite, and Biochar. Furthermore, the introduction of Biochar has significantly increased the Cd content bound to organic matter, with the highest accumulation observed at a 4% Biochar application rate. Various levels of Biochar, Zeolite, and Ca-bentonite have substantially increased the residual Cd, with the highest concentration found in the 4% Biochar treatment.

The study underscores that while an increase in Cd levels diminished the 1000-grain weight in wheat, the incorporation of amendments – Zeolite, Ca-bentonite, and Biochar – mitigated Cd toxicity and concurrently increased the 1000-grain weight. The augmentation in wheat grain weight is attributed to the amelioration of physical, chemical, nutritional, and biological properties of the soil resulting from the presence of Biochar, Zeolite, and Ca-bentonite. Notably, both organic and inorganic amendments demonstrated efficacy in Cd immobilization, but Biochar, as an organic amendment, exhibited a higher immobilizing capacity compared to inorganic amendments. Zeolite and Biochar, known for their robust cation-exchange capacity, play a pivotal role in adsorbing Cd ions, thereby reducing Cd concentration in the soil solution and limiting its bioavailability to plants and other organisms. Zeolites, in particular, alter the distribution of Cd across distinct soil fractions, influencing its presence in exchangeable, carbonate-bound, Fe-Mn oxide-bound, organic matter-bound, and residual forms. The study emphasizes the effectiveness of organic amendments in immobilizing metals due to their high cation exchange capacity and organic carbon content, surpassing the performance of mineral amendments. Additionally, the enduring nature of Biochar, resistant to degradation by soil microorganisms, substantiates its significance in long-term metal immobilization strategies. In general, considering

the performance in reducing cadmium activity, along with environmental impact, cost, abundance, and availability of treatment materials, this study introduces Biochar treatment as the best option. The slow decomposition rate of amendments proves advantageous, ensuring prolonged metal retention. Consequently, from an economic standpoint, the judicious application of soil amendments emerges as a practical and advantageous approach, eliminating the necessity for excessive use.

**Author contributions** All authors contributed to the conceptualization and design of the study. M.R., E.E., and M.O. performed material preparation, data collection, and analysis. A.A. wrote the first draft of the manuscript, edited, and proofread by M.O. and B.B.B. Figures were prepared by M.R. and B.B.B. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Meisam Rahimi, Eisa Ebrahimi and Mohammadreza Ojani. The first draft of the manuscript was written by Ali Adelinia, edited, proof read by Mohammadreza Ojani and Boglárka Bertalan-Balázs. Figures were prepared by Meisam Rahimi and Boglárka Bertalan-Balázs. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Declarations

**Competing interests** The authors declare no competing interests.

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