Environmental Science and Pollution Research (2022) 29:17476–17486
https://doi.org/10.1007/s11356-021-17073-6

RESEARCH ARTICLE

Vermicompost and biochar can alleviate cadmium stress through minimizing its uptake and optimizing biochemical properties in *Berberis integerrima* bunge

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Received: 18 June 2021 / Accepted: 12 October 2021 / Published online: 19 October 2021

Abstract

Organic substrates are gaining popularity as a means of mitigating the negative effects of cadmium (Cd) stress on plant growth. The aim of the present study was to investigate the physio-biochemical attributes of *Berberis integerrima* bunge under Cd-contaminated soil. The pot experiment was carried out based on a completely randomized design (CRD) with six replicates. Cd stress was used as cadmium chloride ($CdCl_2$) at 10, 20, and 30 mg Cd kg^{-1} dry soil. Biochar was applied at the doses of 125 g per pot, and vermicompost was used at the doses of 250 g per pot separately, and for their combination, they were used as 125 g per pot of BC + 250 g per pot of VC. The results showed higher Cd accumulation in both roots and leaves when the soil was polluted with Cd concentrations, but both BC and VC decreased the Cd accumulation in plant tissues. Although chlorophyll content and relative water content (RWC) decreased at 20 and 30 mg Cd kg^{-1} soil, BC and VC, particularly their combination, increased these traits. The highest total phenolic content (TPC) was observed in plants exposed to 20 mg Cd kg^{-1} soil and combined BC and VC. The total flavonoid content (TFC) was increased to 20 mg Cd kg^{-1} soil and then decreased to 30 mg Cd kg^{-1} soil. In addition, organic fertilizer promoted the plants' high accumulation of TFC. The greater activities of antioxidant enzymes including superoxide dismutase (SOD) and phenylalanine ammonia-lyase (PAL) were observed at 30 mg Cd kg^{-1} soil when organic substrates were added. The present study suggests the use of combined BC and VC lead to alleviate the adverse effects of Cd stress in *B. integerrima*.

Keywords Antioxidant enzyme capacity · Heavy metal · Organic substrates · Phenolic content

Introduction

Soil heavy metal contamination is an environmentally important issue due to the increase in anthropogenic activities, volcanic eruptions, atmospheric deposition, and weathering of parent material (Li et al. 2019). Cadmium (Cd), chromium (Cr), mercury (Hg), and arsenic (As) are categorized as high-risk elements for human beings and plants, and they cause deterioration impacts when they exceed their normal concentrations (Rahman and Singh 2019; Zwolak et al. 2019). Cd is a nonessential and non-biodegradable element with adverse effects on ecosystems, uptake of essential plant elements, and soil quality, which thus limits plant growth (Majeed et al. 2021). Cd accumulation in plant tissues can induce physico-biochemical disorders through chlorosis, necrosis, and ion homeostasis, and it may reduce chlorophyll biosynthesis, macronutrient and micronutrient uptake, net photosynthetic activity, and ultimately decline growth and yield (Rizwan et al. 2018; Chen et al. 2020). The excess of Cd may cause severe oxidative damage in plants through the over-production of reactive oxygen species (ROS), which may cause destruction of bio-macromolecules and biomembranes through

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oxidation of lipids and proteins, and may disrupt the antioxidative defense system (Bari et al. 2019).

For detoxification of heavy metals in soils, there are advanced remediation techniques, such as immobilization and inhibition of heavy metal uptake by plants through soil amendments (Dhaliwal et al. 2020). Vermicompost (VC) is a kind of humus-like organic product obtained from the digestive system of earthworms (Bhat et al. 2018). Compared with compost or commercial potting mixture amendments, VC is an excellent organic material for soil fertility due to its desirable properties, including high nitrogen content, high potential for cationic exchange, acceptable porosity, appropriate aeration, drainage, good water-holding capacity, and excellent microbial activity (Singh and Bhartiya 2021; Feizabadi et al. 2021). In addition, the humic acid of VC fortifies it to change the fraction distribution of heavy metals, and possesses carboxylic acids in the molecular structure that respond to protons exchange processes between weak organic acids and heavy metal cations. Therefore, VC is known as an environmentally-friendly soil amendment (Wang et al. 2018). Biochar (BC) is coal produced from plant biomass and agricultural waste that is decomposed in the presence or absence of oxygen. Due to its slow decomposition rate compared with other organic materials, it has a large capacity to reduce greenhouse gases such as carbon dioxide and methane released from waste, and can store carbon for long periods. From an agricultural perspective, one of the advantages of BC is the management of agricultural waste. Biochar acts as an adsorbent for the removal of heavy metal ions including Cd, Pb, Zn, and Cu from the aqueous phase. The BC improves soil fertility through reducing toxic metal bioavailability by adsorption and in situ stabilization due to its alkaline nature, electronegative charge density, porosity, and large surface area (Wang et al. 2018; Majeed et al. 2021). However, the soil amelioration ability of BC strongly depends on soil properties, plant and feedstock type, rate of application, and pyrolytic temperature (Huang et al. 2021). Biochar increased the stomatal conductance, water use efficiency, chlorophyll contents, photosynthetic, and relative water content of drought-stressed tomato leaves (Akhtar et al. 2014). Meanwhile, Cui et al. (2020) indicated that biochar application decreased Pb (40.0%) and Cd (84.1%) transfer from root to rice grain. Compared to the control, biochar application increased the leaf RWC, osmotic potential, and transpiration rate of drought-stressed maize (Haider et al. 2015).

Berberis integerrima bunge is a deciduous shrub of the Berberidaceae family, up to 5 m tall with obovate leaves, yellow wood, yellow flowers, and red fruits. It grows widely in the Middle East and central parts of Asia (Azimi et al. 2018). *B. integerrima* is used as an ornamental shrub in green spaces and urban areas, and the fruit is used in the food industry in the preparation of jellies, candies, juices, and marmalades (Tavakoli et al. 2017).

There is an increasing interest in the application of organic materials to alleviate the adverse effects of Cd stress in agricultural and horticultural systems (Wang et al. 2018). In many parts of the Middle East, such as Iran, the pruned leaves of the date palm consist of valuable materials that need to be processed and handled better. In addition, VC, a popular organic fertilizer, needs to be widely used on Cd-contaminated soils. Several works have demonstrated the positive effect of BC on mitigating Cd toxicity and improving growth by changing the physiological and biochemical attributes of *Mentha arvensis* (Nigam et al. 2019), *Oryza sativa* (Rizwan et al. 2018), *Spinacia oleracea* (Younis et al. 2016), *Triticum aestivum* (Abbas et al. 2017), *Oryza sativa* (Rizwan et al. 2018), and *Brassica napus* (Kamran et al. 2020).

Although the positive effect of VC and BC has been pronounced on plant growth under salinity and drought has been frequently studied (Hafez et al. 2020; Ebrahimi et al. 2021), the effects of BC and VC alone or in combination to inhibit or mitigate Cd stress in *B. integerrima* bunge, remain poorly understood. In addition, biochemical attributes such as antioxidant capacity and phenolic contents of *B. integerrima* bunge have not yet been investigated under these treatments. Therefore, the present study aimed to assess the potential of BC and VC only and their combination on growth, antioxidant capacity, phenolic, and water content of *B. integerrima* plants in the Cd-polluted soil.

Material and methods

Plant materials and growth conditions

The 3-year seedlings of *B. integerrima* bunge were prepared from a nursery in Karaj, Iran (1314 m asl, 35°48′42″ N, 51°01′33″ E). The seedlings were transferred to the 5-L pots in a greenhouse with a relative humidity of 60–70% and a photoperiod of 16 h (lightness) and 8 h (darkness) at the University of Tehran, Karaj, Iran. Each pot contained one plant of *B. integerrima*. The sowing date of *B. integerrima* was 10 April 2020. The soil was sandy loam with a pH: 7.1, EC:1.1 dS m^{-1}, N: 0.27%, P: 13.2 mg kg^{-1}; K:217 mg kg^{-1}; and total Cd: 0.14 mg kg^{-1}.

Organic substrates

The organic fertilizers used in the experiment were VC and BC. Vegetable waste, wheat straw and husk, weeds, and cow dung were used to make the VC. Earthworms (Eisenia fetida) were healthy and allowed to feed on these organic materials for 3 months to create VC. The BC was created by slowly pyrolyzing date palm branches and leaves at 560 °C for 4 h at atmospheric pressure in a mobile ring kiln. Table 1 shows the physio-chemical attributes of VC and BC.

Experimental design and treatments

The factorial experiment with organic fertilizers and Cd stress was carried out based on a completely randomized design (CRD) with six replicates. Cadmium chloride ($CdCl_2$) (Sigma–Aldrich, USA) was used to exert Cd stress at concentrations of 10, 20, and 30 mg Cd kg^{-1} dry soil. Biochar was applied at the doses of 125 g per pot, and vermicompost was used at the doses of 250 g per pot separately, and for their combination, they were used as 125 g per pot of BC + 250 g per pot of VC. The doses of BC and VC were chosen based on results with these organic substrates in our previous studies. Due to the difference in the texture and density of BC and VC, their amount should be different as soil amendments. This difference of BC and VC has been previously reported in most articles so that BC mass has been lower than VC. For each treatment, the soil was completely mixed with corresponding factors of Cd and organic materials. After 5 months of planting, the aerial parts of plants were harvested at the end of the growing stage and air-dried for further analysis.

Cadmium concentration of leaves and roots

To measure total Cd concentration in leaves and roots, after digesting 500 mg of the sample with 10 mL of $HNO_3/HClO_4$ (4:1, v/v), it was determined by an atomic absorption spectrophotometer (Shimadzu AAS-6300 model, Japan) (Qin et al. 2017).

Fresh and dry weight

In order to measure shoot and root fresh and dry weight, the above and belowground of *B. integerrima* plants were harvested at the flowering stage. The shoot and root tissues were pulled up, and the dry weight was recorded after drying in an oven at 70 °C until they got a constant weight to determine the dry weight (Saki et al. 2019). The sample weight was recorded on a digital scale (±0.001 g).

Table 1 Characteristics of biochar and vermicompost used in the experiment

	Vermicompost	Biochar	Soil
pH	7.5	8.4	7.8
EC (dS m^{-1})	2.1	6.5	1.2
N (%)	2.1	0.9	0.55
K (g kg^{-1})	28.5	8.2	135
P (g kg^{-1})	1.8	1.5	9.7
Ca (g kg^{-1})	44.5	6.5	5.9
Mg (g kg^{-1})	10.4	4.5	1.7
Cd (g kg^{-1})	-	-	0.47

Relative water content

The developed fresh leaves were used to measure RWC. Leaf samples were quickly transferred to the laboratory on ice cubes, and their fresh weight (FW) was measured. The leaves were then soaked in distilled water for 24 h at room temperature and under low light to determine the saturation weight, after which the samples were rapidly and accurately weighed with a dry paper towel and their saturation weight (SW) calculated. Finally, the samples were dried in an oven at 70 °C for 24 h to determine the dry weight (DW), and the RWC was determined using the following equation (Dhopte and Manuel 2002).

$$RWC = \frac{(FW - DW)}{(SW - DW)} \times 100$$

Chlorophyll assay

For chlorophyll content determination, the fifteen developed leaves were harvested from the plants. Leaves were cleaned with deionized water to remove any surface contamination. According to Arnon (1949), the contents of Chl a and Chl b were measured from fresh leaves. In 8 mL of 80% acetone, 200 mg of fresh samples were homogenized. After that, the mixture was centrifuged for 15 min at 4 °C (3000 rpm). The chlorophyll concentration of supernatants was determined. The spectrophotometer measured absorbance at 645 and 663 nm. Chlorophyll content was calculated using the below equation:

$$\text{Chlorophyll a} = (9.93 \times A663 - 0.77 \times A645) \times V/W \times 1000$$
$$\text{Chlorophyll b} = (17.6 \times A645 - 2.81 \times A663) \times V/W \times 1000$$

Total phenolic content

The TPC was calculated spectrophotometrically using the Folin–Ciocalteu reagent (McDonald et al. 2001). The Folin–Ciocalteu reagent was combined with 4 mL of Na_2CO_3 (1 M) solution in a volume of 5 mL. After that, 0.5 mL of plant extract or gallic acid (GA) was added to the mixture. The mixes were left at room temperature for 15 min. Subsequently, a Lambda 45-UV/Visible spectrophotometer was used to determine the absorbance of the samples. The standard curve was created using concentrations of

GA. The TPC was shown as mg of GA per g of dry weight (DW).

Total flavonoid content

According to Chang et al. (2002), the TFC was determined using aluminium chloride.

First, 0.1 mL of aluminium chloride (10%) was mixed with 0.1 mL of potassium acetate (1 M), followed by 2.8 mL of distilled water. Next, 0.5 mL of each extract was added to the combination of aluminium chloride, potassium acetate, and water, along with 1.5 mL of ethanol. The final mixture (with a volume of 5 mL) was allowed to incubate at room temperature for 30 min. A Lambda 45-UV/Visible spectrophotometer was used to evaluate the adsorption of the reaction mixture at 415 nm. TFC was measured in milligram of quercetin (QE) per gram of dry weight.

Superoxide dismutase activity

The leaves at physiological maturity (56 days after transplanting) were used to determine SOD activity as described by Beauchamp and Fridovich (1971). The 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M KCN, and 5.6×10^{-5} M nitroblue tetrazolium salt (NBT) were dissolved in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8). Three millilitre of the reaction was added to 1 mL of enzyme extract. Illumination was started to activate the reaction at 30 °C for 60 min. The blanks were identical solutions kept under dark conditions. The absorbance was determined at 560 nm with the spectrophotometer against the blank.

Phenylalanine ammonia-lyase activity

The developed leaves were harvested at the flowering stage. By measuring the synthesis of t-cinnamic acid at 290 nm in developed leaves, the PAL activity was determined (Hahlbrock and Ragg 1975). The 50 mmol Tris–HCl buffer (pH 8.8), 20 mmol L-phenyl alanine, and enzyme extract were used in the reaction. The reaction was halted with the addition of 0.5 mL of 10% trichloroacetic acid after 30 min of incubation. After 30 min, the absorbance at A nm was measured. The amount of enzyme that causes a 0.01 per min decrease in absorbance was defined as one unit of enzyme activity. The activity of the PAL enzyme was measured as enzyme units per gram of fresh weight (U/g FW).

Statistical analysis

SAS software for Windows was used to analyse the data (SAS, version 9.2, SAS Institute, Cary, NC). Duncan's multiple range tests were used to find the mean comparison. All data were statistically analysed at a 5% probability level ($P \leq 0.05$).

Results

Cadmium concentration in leaves and roots

The Cd content in leaves and roots was significantly affected by the nitration of Cd stress and organic fertilizers ($P \leq 0.05$) (Fig. 1). The Cd content in both leaves and roots increased by progressing its levels, whereas organic fertilizers prevented Cd accumulation in plant tissues (Fig. 1). The highest Cd accumulation was reported in plants exposed to 30 mg Cd kg^{-1} soil and non-fertilizer application. Both BC and VC played a significant role in mitigating the Cd accumulation in plant tissues. In high Cd stress level (30 mg Cd kg^{-1} soil), BC, VC, and their combination alleviated Cd accumulation in leaves by 50, 52, and 65%, respectively, compared to control (non-organic fertilizer) (Fig. 1a). These mitigations were 41, 46, and 61%, respectively, for roots (Fig. 1b).

Plant weight

Table 2 shows the changes in shoot and root weight of *B. integerrima* plants under Cd stress and organic substrates. Both the fresh and dry weights of the shoot were significantly changed under cadmium and organic fertilizers ($P \leq 0.05$). The fresh and dry weights of aerial parts decreased by progressing Cd concentration. High Cd levels (30 mg kg^{-1} soil) decreased shoot fresh and dry weight by 42 and 43%, respectively, as compared with control (Table 2). Like shoot, there was a significant reduction in the root weight of plants experiencing 20 and 30 mg Cd kg^{-1} soil concentrations. However, we obtained no significant changes in root weight between control and slight Cd stress (10 mg Cd kg^{-1} soil). The effect of organic fertilizers was significant on the root and shoot weight of *B. integerrima* plants ($P \leq 0.05$). The combined BC and VC were more effective in improving the plant weight, where this treatment increased the fresh and dry weight of plants by 34 and 33%, respectively, compared with non-treated plants (control). This improvement for fresh and dry root weight was respectively 18 and 20% relative to control.

Photosynthesis pigments

The interaction of Cd stress and organic fertilizers significantly affected Chl a content ($P \leq 0.05$) (Fig. 2). The 20 and 30 mg Cd kg^{-1} soil remarkably reduced Chl a, but the organic fertilizers enhanced it (Fig. 2a). Compared to the non-fertilizer application (control), the 20 and 39% reductions in Chl a were observed with 20 and 30 mg Cd kg^{-1}

Fig. 1 Cadmium (Cd) concentration in leaves (**a**) and roots (**b**) of *Berberis integerrima* bunge plants under Cd stress and organic amendments (vermicompost, VC; biochar, BC). Values are means ± standard deviation (SD) of three replications ($n = 3$). Different letters show statistically significant differences among treatments at $P \leq 0.05$

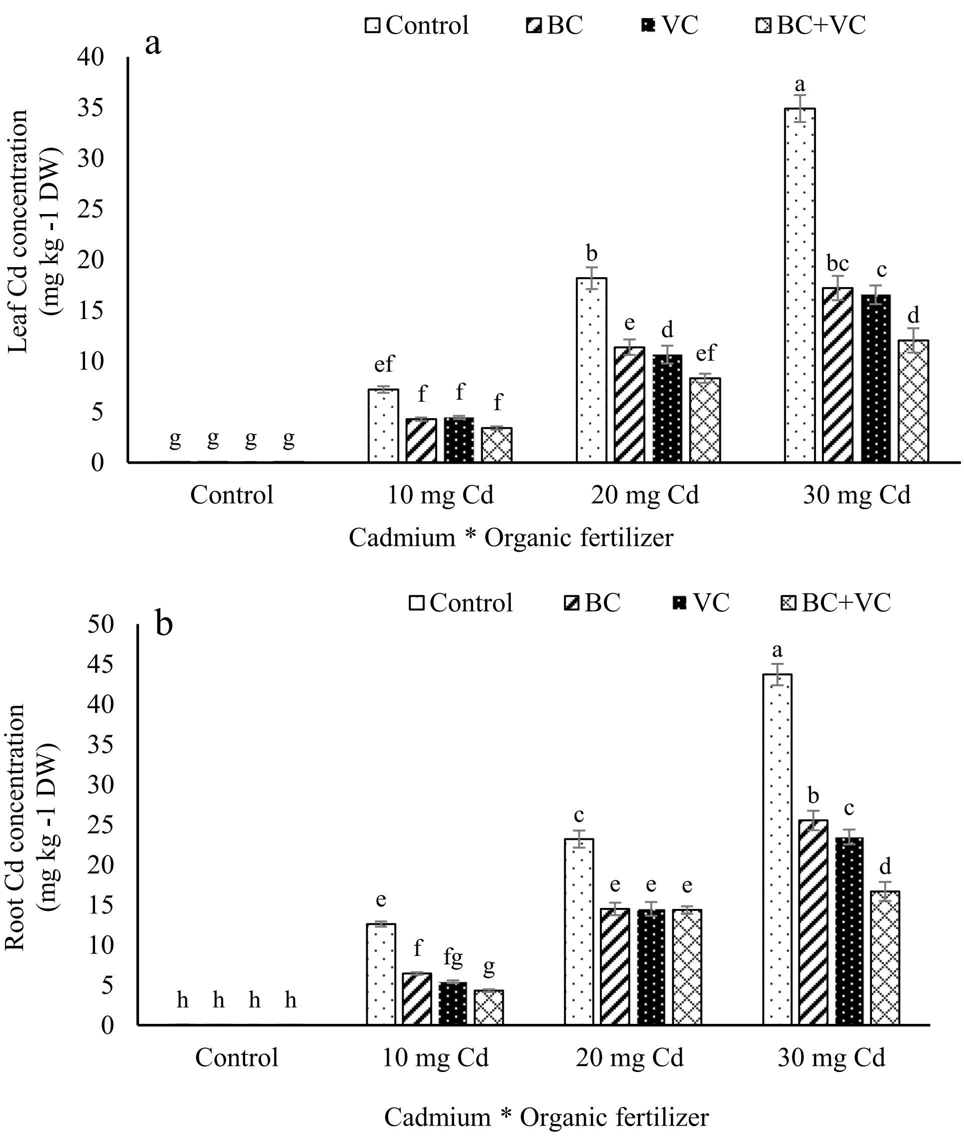

Table 2 Shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) of *Berberis integerrima* plants under cadmium stress and organic fertilizers

Treatments	SFW	SDW	RFW	RDW
Cadmium (mg kg^{-1} soil)				
Control	10.1 ± 1.02^{a}	3.88 ± 0.49^{a}	10.27 ± 0.52^{a}	4.37 ± 0.27^{a}
10 mg	9.7 ± 1.27^{b}	3.85 ± 0.35^{a}	10.93 ± 0.92^{a}	4.42 ± 0.43^{a}
20 mg	6.6 ± 0.69^{c}	2.50 ± 0.27^{b}	8.45 ± 0.51^{b}	3.51 ± 0.23^{b}
30 mg	5.8 ± 0.77^{d}	2.18 ± 0.34^{c}	6.06 ± 1.71^{c}	2.61 ± 0.75^{c}
Organic fertilizer				
Control	6.83 ± 0.69^{c}	2.63 ± 0.24^{c}	8.22 ± 0.65^{b}	3.40 ± 0.22^{a}
Biochar (BC)	8.05 ± 0.78^{b}	3.11 ± 0.28^{b}	9.03 ± 0.72^{ab}	3.78 ± 0.19^{ab}
Vermicompost (VC)	8.25 ± 0.72^{b}	3.16 ± 0.27^{b}	8.69 ± 0.78^{b}	3.65 ± 0.24^{bc}
BC + VC	9.16 ± 0.82^{a}	3.51 ± 0.32^{a}	9.77 ± 0.86^{a}	4.07 ± 0.32^{a}

soil (Fig. 2a). However, Chl a increased when organic fertilizers were added. Under high Cd stress, BC, VC, and their combination improved the Chl a content by 34, 26, and 21%, respectively, compared with control (Fig. 2a). Chl b decreased by progressing Cd stress, as its minimum amount (0.24 mg g^{-1} FW) was obtained at 30 mg Cd kg^{-1}

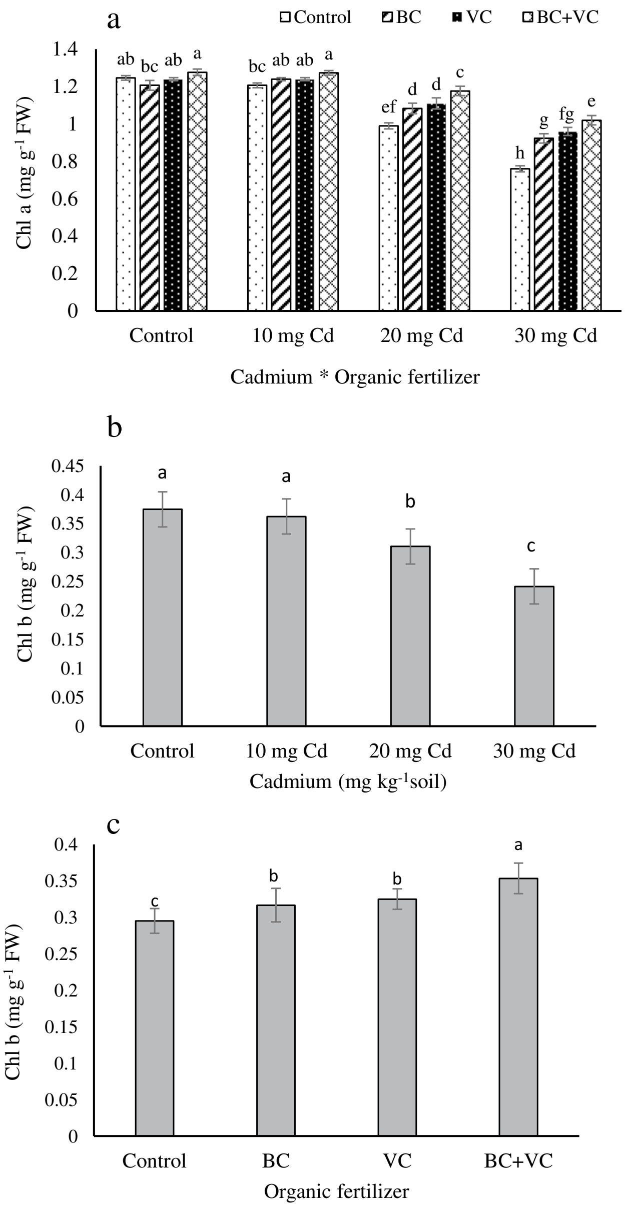

Fig. 2 Chlorophyll a (Chl a) (**a**) and chlorophyll b (Ch b) (**b**, **c**) content of *Berberis integerrima* bunge plants under Cd stress (10, 20 and 30 mg) and organic amendments (vermicompost, VC; biochar, BC). Values are means ± standard deviation (SD) of three replications ($n=3$). Different letters show statistically significant differences among treatments at $P \leq 0.05$

soil (Fig. 2b). Unlike Cd stress, organic fertilizer improved Chl b, in which its highest amount was obtained in the combination of BC and VC to be 0.35 mg g^{-1} FW (Fig. 2c).

Relative water content

The RWC decreased with Cd stress, but increased with organic fertilizers ($P \leq 0.05$) (Fig. 3). The highest (74.4%) and lowest (59.7%) RWC were observed in non-stressed plants and 30 mg Cd kg^{-1} soil, respectively (Fig. 3a). Nevertheless, organic fertilizers improved RWC. The BC and VC only or in combination had a significant role in increasing RWC, as its maximum amount was obtained in plants treated with the combined BC and VC of 71.40% (Fig. 3b).

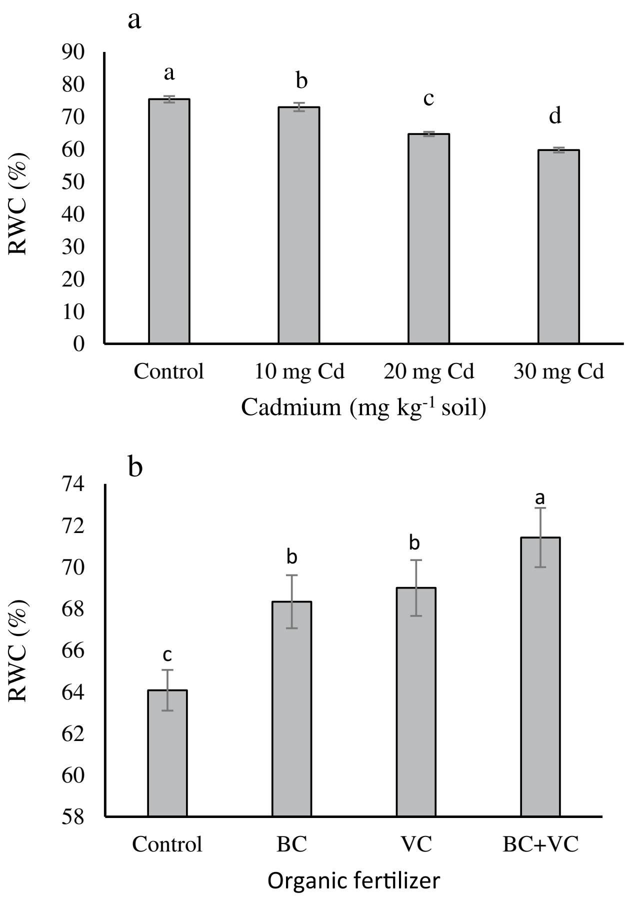

Fig. 3 Relative water content (RWC) of *Berberis integerrima* bunge plants under Cd stress (**a**) and organic amendments (vermicompost, VC; biochar, BC) (**b**). Values are means ± standard deviation (SD) of three replications ($n=3$). Different letters show statistically significant differences among treatments at $P \leq 0.05$

Total phenolic content and total flavonoid content

The interaction of Cd stress and organic fertilizer was significant on TPC ($P \leq 0.05$) (Fig. 4a). The TPC increased up to 20 mg Cd kg^{-1} soil, and then decreased (Fig. 4a). All organic fertilizers improved TPC, and their effects were demonstrated when exposed to 20 and 30 mg Cd kg^{-1} soil. Under 20 mg Cd kg^{-1} soil, BC, VC, and their combination increased TPC by 15, 13, and 21%, respectively, compared with control (Fig. 4a). Both Cd stress and organic fertilizer significantly changed TFC ($P \leq 0.05$). The highest TFC was obtained at 20 mg Cd kg^{-1} soil, while its lowest amount was observed in control to be 11.33 mg QE g DW (Fig. 4b). The

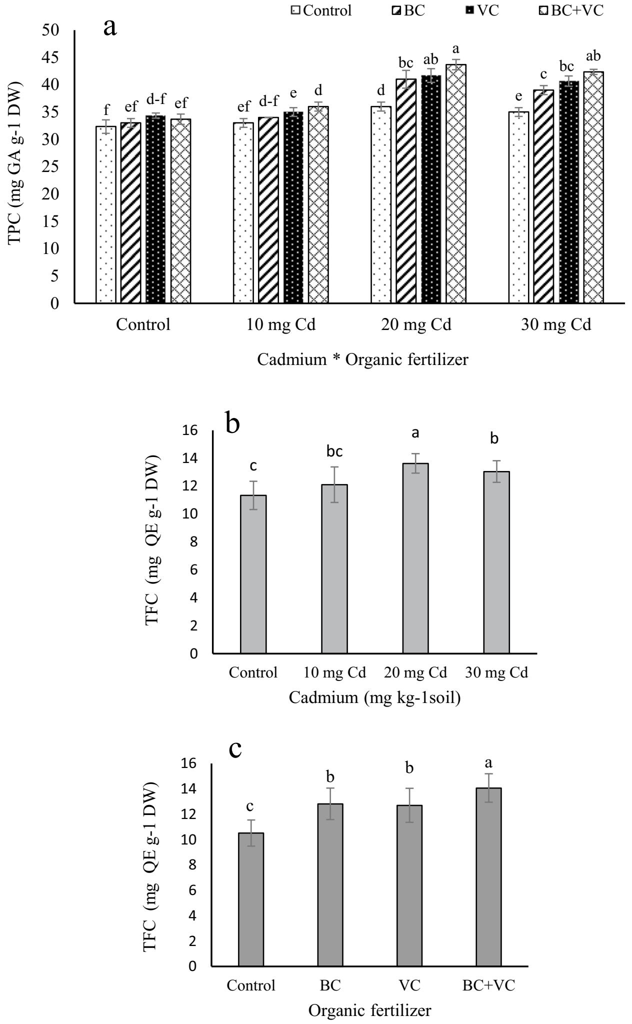

Fig. 4 Total phenolic content (TPC, **a**) and total flavonoid content (TFC, **b** and **c**) of *Berberis integerrima* bunge plants under Cd stress and organic amendments (vermicompost, VC; biochar, BC). Values are means ± standard deviation (SD) of three replications ($n = 3$). Different letters show statistically significant differences among treatments at $P \leq 0.05$

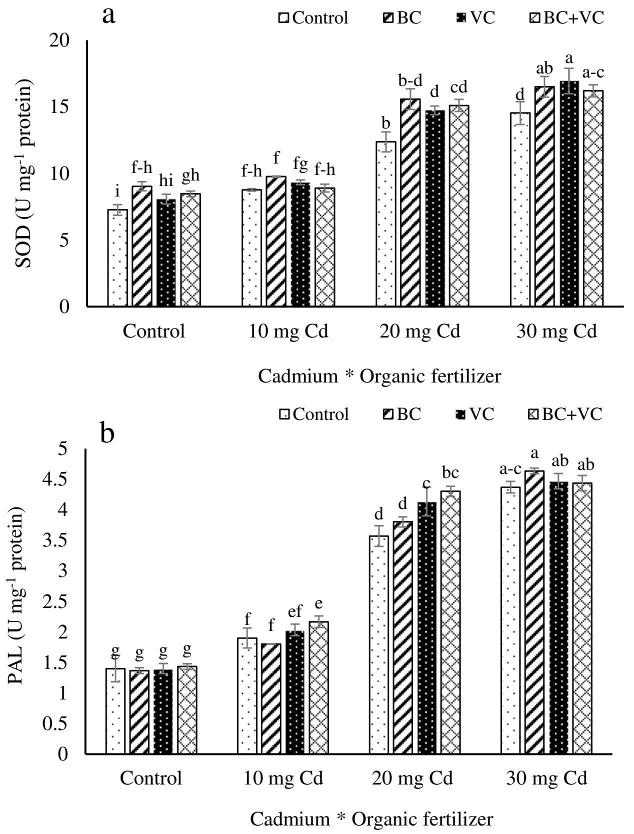

Fig. 5 Superoxide dismutase (SOD, **a**) and phenylalanine ammonia-lyase (PAL, **b**) activity of *Berberis integerrima* bunge plants under Cd stress and organic amendments (vermicompost, VC; biochar, BC). Values are means ± standard deviation (SD) of three replications ($n = 3$). Different letters show statistically significant differences among treatments at $P \leq 0.05$

TPC increased by the only combination of BC and VC, in which its maximum received at combined BC and VC was 14.06 mg QE g DW (Fig. 4c).

Superoxide dismutase and phenylalanine ammonia lyase activities

The activity of SOD and PAL was significantly changed under the interaction of Cd stress and organic fertilizer ($P \leq 0.05$) (Fig. 5). SOD activity was enhanced by increasing Cd concentration (Fig. 5a). In non-fertilizer treatment, we obtained a twofold increase in SOD activity in high Cd-polluted soil compared to control. The highest SOD activity was reported in plants exposed to high Cd stress and VC to be 16.9 U mg^{-1} protein (Fig. 5a). Like to SOD, PAL was increased by Cd stress and organic fertilizers. Under non-fertilizer application, 20 and 30 mg Cd kg^{-1} soil increased PAL activity by 2.5- and threefold (Fig. 5b).

Discussion

The results noticeably represented an increased accumulation of Cd in *B. integerrima* plants when exposed to Cd stress, in which its concentration in roots (up to 43.7 mg kg^{-1} DW) was higher than in leaves (up to 34.9 mg kg^{-1} DW). Like our results, Gheshlaghpour et al. (2021) reported higher Cd accumulation in roots relative to aerial parts of basil. The increased Cd concentration in the soil causes acidification of the rhizosphere and leads to enhancement of Cd bioavailability and mobility. Plants, via their phytoremediation process, accumulate Cd in their tissues, and ultimately contaminate the food chain, which is dangerous for human and animal health (Rahman and Singh

2019). Therefore, reliable techniques to mitigate Cd toxicity and minimize Cd accumulation in plants are necessary. It has been documented that Cd stimulates overexpression of the *HMA*2 gene, resulting in hyper-accumulation of Cd in different tissues (Gheshlaghpour et al. 2021). The positive function of BC, VC, and their combination as effective practice to mitigate the Cd accumulation of *B. integerrima* was demonstrated in this study. Organic substrates reduce Cd bioavailability and mobility in the soil, restrict Cd absorption and translocation, sequester Cd in the vacuole, and regulate transpiration rate, all of which help plants tolerate Cd stress (Guo et al. 2018). BC can improve the chemical properties of soil, like pH and electrical conductivity (EC). Soil pH improvement by BC is to cover the pH gap between soil and organic material (Albert et al. 2021a, b). Cd concentrations were shown to be lower in the shoot and root of Solanum nigrum, as well as in the available Cd concentration when VC was added (Yang et al. 2019).

Plant biomass, as an indicator of plant growth, was decreased by Cd stress (Table 2). Cd is a non-essential and hazardous trace element for plants, and its accumulation in plant tissues can prevent photosynthesis and growth by disrupting normal physiological and molecular functions through increased ROS generation (Rizwan et al. 2018). BC, VC, and particularly their combination can improve the shoot and root biomass, which can be related to the positive roles of BC and VC in soil physicochemical properties to reduce Cd availability (Wang et al. 2018). Similar to our results, Álvarez et al. (2018) reported the increased plant weight of *Pelargonium peltatum* and *Petunia hybrida* treated with combined BC and peat-based materials.

The rise in shoot and root weight is likely due to BC and VC's role in improving mineral availability, particularly P, which promotes root growth and nutrient uptake (Ebrahimi et al. 2021). Plant growth is aided by a combination of mineral bioavailability and plant hormones, such as auxins and gibberellins (Souri and Sooraki 2019). Similarly, the positive role of VC and BC application in improving root and shoot biomass has been previously reported in different studies (Nazarideljou and Heidari 2014; Alvarez et al. 2017). BC and VC can change soil attributes and create suitable conditions for plant growth. Both BC and VC improve soil texture, porosity, structure, and density, and particle size distribution. BC due to its high porosity and more surface area can provide desirable conditions for microorganisms beneficial for the soil and may increase cation exchange capacity (CEC) through the binding of important anions and cations (Ebrahimi et al. 2021). Increased soil pH with BC application leads to improved availability of minerals, especially phosphorous and potassium (Alvarez et al. 2017(. The soil application of BC causes an oxidation process on the particle surface. Increased CEC aids lead to improved soil fertility, as the nutrients will remain attached to the soil, opposing the leaching process because of CEC (Souri et al. 2019). VC releases more nitrogen into the soil, and it results in higher biomass in plants. Singh and Varshney (2013) reported increased NH_4^+-N and NO_3^--N in soil when VC was added. The VC can improve the growth of nitrogen-fixing microorganisms in the rhizosphere, which enhances N availability by making biologically fixed N available through the intimate mixing of ingested particles with soil (Wanget al. 2017). Similar to our results, the increased biomass of tomato has been reported by Wang et al. (2017). When the combined BC and VC were used, the better biomass was observed (Table 2), and it could be due to the different positive effects of each organic material, which exerted on plant growth when applied together.

Chl content and RWC decreased with Cd application, but increased with organic materials (Figs. 3 and 4). The decline of Chl and RWC was the strategy of *B. integerrima* to cope with the Cd-stressed conditions. Cd stress can limit the photosynthesis rate in plants. Under stress conditions, there are two main limitations to the photosynthesis process. The first is the stomata limiting factor, which is associated with stomatal closure and reduces CO_2 concentration in levees and its transfer to the chloroplast, ultimately limiting photosynthesis. The second is a non-stomatal limiting factor, which includes a decrease in rubisco content and activity, inhibition of ribulose-1, 5-bisphosphate synthesis, and a decrease in photosynthetic electron transport to the PSII (Amiri et al. 2017). ROS activity under Cd stress leads to lipid oxidation; changes in protein structure; oxidation of sulfhydryl groups (-SH) in proteins and their inactivation; bleaching or loss of pigments, such as chlorophyll and carotenoid; and attacks photosystems (Zaid and Mohammad 2018). The VC and BC due to their high water-holding potential and improvements in physical and chemical properties of soil improve mineral uptake by roots, which results in a higher photosynthesis rate (Houshmandfar et al. 2018). The main elements involved in photosynthesis, particularly P, release into the rhizosphere in the presence of organic materials (Arancon et al. 2019).

RWC, which is defined as a physiological response in plants to stressful plants, can be used to assess plant water status (Sarker and Oba 2018). The plants subjected to moderate and severe Cd stress had a lower RWC (Fig. 4) (Sarker and Oba 2018).

Reduced RWC in the leaves is caused by a decrease in turgor pressure, water uptake limitation in available water, and the prohibition of water uptake by roots under stress conditions. A decline in RWC in plant tissues is a strategy used by plants to alleviate some biochemical processes by conserving energy for survival (Sarker and Oba 2018). Organic substrates create a desirable environment for plants by increasing water content, which improves biochemical processes in plant cells and, therefore, plant growth and yield (Merwad et al. 2018). Similar to our results, Feizabadi et al. (2021) indicated increased RWC of different canola cultivars after using VC.

Different roles of phenolic acids as secondary metabolites have been identified; they play a principal role in plant adaptation to the environment and stress resistance (Kısa et al. 2016). A moderate concentration of Cd stress improved TPC and TFC, while severe Cd stress decreased these compounds. TPC and TFC are both involved in many stress responses and accumulate in plant tissues when plants are stressed (Saki et al. 2019). The phenolic metabolites in heavy metal contaminated soil act as metal chelators and engage in ROS scavenging (Malčovská et al. 2014). The accumulated TPC and TFC under Cd stress have been addressed in different tissues of plants (Malčovská et al. 2014; González-Mendoza et al. 2017). Phenolic compounds are formed via the shikimic acid or phenylpropanoid pathways, and as hydroxyl groups can donate hydrogen and react with ROS in the termination reaction, the cycle of producing new radicals is broken (Gharibi et al. 2016). VC can play an important role in use of organic manufacturing systems to improve the flavonoid biosynthesis in plants (Bakhtiari et al. 2020). Several documents have shown the positive effect of VC on increasing the synthesis of flavonoids in plants (Gholami et al. 2018; Bakhtiari et al. 2020). The application of organic substrates improves the plant's ability to scavenge free radicals by enhancing phenolic compounds (Bakhtiari et al. 2020). This elucidates the role of BC and VC in compound biosynthesis to motivate the high production of shikimic acid, causing more production of phenolic compounds such as flavonoids (Bakhtiari et al. 2020).

Antioxidant enzymes have a significant role in protecting plants from heavy metal pollution (Khosropour et al. 2019). Increased SOD and PAL activity were reported in the presence of both Cd and organic fertilizers (Fig. 5). The SOD is activated as the first line of antioxidant system defense against the ROS produced under stressful conditions. It catalyses super-oxide anion radicals to H_2O_2 and water (Zuo et al. 2018). Pan et al. (2020) showed increased SOD activity at Cd stress conditions in *Kandelia obovata*. Under Cd stress, the increased expression of genes involved in SOD and PAL can be observed, which is expressed as a higher activity of these enzymes to cope with the undesirable conditions that occur for plants. The increased activity of antioxidant enzymes has been reported in strawberry plants in the presence of VC (Zuo et al. 2018), which is consistent with our results.

Conclusion

The present study showed that 20 and 30 mg Cd kg^{-1} soil significantly decreased growth, RWC, and Chl content of *B. integerrima*; 10 mg Cd kg^{-1} soil had no remarkable differences in physiological and biochemical properties compared with control. The combined VC and BC as organic amendments play an effective role in improving the biochemical status of this plant. Increased phenolic content up to 20 mg Cd kg^{-1} soil and antioxidant enzymes in moderate and high stress are the biochemical strategies of *B. integerrima* to cope with Cd stress by helping organic materials. Therefore, according to our results, we suggest the mixed application of VC and BC to alleviate the adverse effects of Cd stress. Meanwhile, further studies are needed in order to understand the mechanism by which VC and BC affects several groups of secondary metabolites, and antioxidant enzymes activity in different plant species, in order to gained insights into understanding of role of these organic substrates in agricultural production. Furthermore, more studies on various VC and BC doses should be employed to help in the reduction of Cd toxicity.

Author contribution Esmail Khosropour: conceptualization, methodology, reviewing; Weria Weisany: data curation, writing—original draft preparation, software; Nawroz Abdul-razzak Tahir, Leila Hakimi: reviewing and editing.

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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