Environmental Technology & Innovation 36 (2024) 103753

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Bamboo biochar helps minimize *Brassica* phytotoxicity driven by toxic metals in naturally polluted soils of four mine zones

Abolghassem Emamverdian [a,*], Abazar Ghorbani [b,c], Necla Pehlivan [d], Yang Li [e], Meisam Zargar [f], Guohua Liu [a,*]

[a] *Co-Innovation Center for Sustainable Forestry in Southern China,.Bamboo Research Institute, Nanjing Forestry University, Nanjing 210037, China*
[b] *National Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Center for R&D of Fine Chemicals of Guizhou University, Guiyang, 550025, China*
[c] *Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Islamic Republic of Iran*
[d] *Department of Biology, Recep Tayyip Erdogan University, Rize 53100, Turkiye*
[e] *Department of Mathematical Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA*
[f] *Department of Agrobiotechnology, Institute of Agriculture, RUDN University, Moscow 117198, Russia*

ARTICLE INFO

Keywords:
Bamboo biochar
Soil amendment
Plant stress tolerance
Toxic metal
Phytoremediation

ABSTRACT

Researchers have recently become interested in utilizing biochar amendment as an organic approach to enhance soil quality and minimize the mobility of toxic metals (TMs), which can help grow TM-tolerant plant species in polluted areas. A pot experiment was conducted to examine the efficacy of bamboo biochar (BB) in reducing the phytotoxicity of four unique mine-contaminated soil types. According to a completely randomized design (CRD), in four replications on *Brassica juncea*, a five-level bamboo biochar treatment (0 % Control, 2.5 % BB, 5 % BB, 7.5 % BB, and 10 % BB) was administered in naturally contaminated areas of Sarcheshmeh, Gol-Gohar, Chadormalu, and Anguran mines. The data show that Bamboo Biochar (BB) increased soil enzymatic activities (58 %), reformed soil structure, including pH (7 %) and electrical conductivity (EC) (51 %), and decreased the availability of TMs (Zn (37 %), Pb(34 %), Cd(51 %), Cu(34 %)), preventing accumulation in roots (42 %) and translocation to shoots (38 %). The phytochelatin (79 %), ascorbic acid (56 %), glutathione contents (57 %), and antioxidant (51 %) and glyoxalase activities (71 %) in *B. juncea* ultimately enhanced root-shoot dry biomass (44 %) and overall tolerance to TMs in mine-polluted soil (43 %). BB at 10 % might be used as a reliable soil amendment and natural metal immobilization adsorbent in the soil, as well as a suitable option for reducing oxidative stress caused by TMs in *B. juncea* plants, which are strong phytoremediation candidates in polluted soils. Future research endeavors might aim to discover cost-effective, efficient, and natural substances that can enhance and diminish environmental toxicity, eliminate soil contamination caused by heavy metals, and ultimately enhance human well-being. Keywords: Biochar Application; Soil amendment; Plant stress tolerance; Toxic metal; Phytoremediation

---

* Corresponding authors.
E-mail addresses: emamverdiyan@njfu.edu.cn (A. Emamverdian), ghliu@njfu.edu.cn (G. Liu).

https://doi.org/10.1016/j.eti.2024.103753
Received 23 April 2024; Received in revised form 12 July 2024; Accepted 14 July 2024

1. Introduction

Toxic metals are one of the most critical environmental pollutants released by significant sources such as extensive urbanization, industrial waste disposal, and mine explorations (Adnan et al., 2022; Emamverdian and Ding, 2017; Emamverdian et al., 2018). Industrial activity is a primary contributor to the accumulation of metals in the environment, and mining projects play a significant role Multiple studies in recent years have found that TM levels have risen around mines through mining activities, particularly at mine waste disposal sites, impacting the environment, the local ecology, plant development, and human health (Dusengemungu et al., 2022; Hlihor et al., 2022). Iran possesses abundant mineral reserves throughout the nation's boundaries, resulting in an extensive accumulation of heavy metals that contaminate a large portion of the country's soil, mainly due to mine tailings (Hosseini et al., 2018). However, insufficient research has been conducted on using native plants as a sustainable approach to remediate the toxicity near mine tailings (Ghaderian and Ghotbi Ravandi, 2012). Toxic metals in soil can affect soil enzymatic activities and soil characteristics such as soil pH, electrical conductivity (EC), and soil organic matter (Xian et al., 2015; Ali et al., 2017). By interfering with gas exchange, photosynthetic pigments, chlorophyll concentration, and stomatal conductance, prolonged TM contamination in soils reduces plant growth and yield (Mourato et al., 2015). Toxic metals also induce plant oxidative stress via generating, e.g., excessive "Reactive Oxygen Species (ROS)" (Georgiadou et al., 2018) or highly reactive derivatives such as methylglyoxal (MG) (Ghorbani et al., 2021, 2022; Raihan et al., 2022). When plants are subjected to high levels of TM stress, toxic substances such as excessive ROS and MG may significantly disrupt plant metabolism and damage cell membranes (Rai et al., 2021; Kharbech et al., 2020). Being anchored and rooted, prolonged contamination may eventually lead to plant death (Zulfiqar et al., 2023; Wu et al., 2021; Emamverdian et al., 2023). However, two crucial glyoxalase system enzymes, glyoxalase I and II, are in charge of MG scavenging in the first line of defense (Hasanuzzaman et al., 2011; Mousavi et al., 2020). Reactive oxygen compounds include free radicals such as hydroxyl radical (•OH) and superoxide anion (O^2•−) as well as non-radical molecules, such as hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) (Sharma et al., 2012; Ghorbani et al., 2024). Plants employ defense mechanisms that cope with environmental stress, which can trigger the activation of antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). This activation leads to reactive oxygen species (ROS) scavenging and protecting plant cells (Sytar et al., 2013). Enzymes function by converting molecules that are by-products of metabolic processes into less harmful molecules, e.g., by facilitating the conversion of H_2O_2 into less harmful oxygen and water molecules. Therefore, increasing the glyoxalase system and antioxidant capacities, such as enhancing the activity of antioxidant enzymes like POD, SOD,CAT, and phenylalanine ammonia-lyase (PAL) protecting plant cells against excessive radical compounds are two fundamental defensemechanisms under stress in TM contaminated soil (Altaf et al., 2023).

In agricultural systems, different materials have been used to reduce the bioavailability and immobilization of TMs which are including phosphate fertilizers, CaO, fly ash, organic waste (compost), medical stone, and biochar (Mahar et al., 2016; Hmid et al., 2015; Wang et al., 2016). Biochar is a carbon-rich substance that is often produced at high temperatures (>300°C) in limited oxygen conditions and can be made from a variety of organic resources, including agricultural and forest residual biomass, wood, straw, and manure (Gonzaga et al., 2017; Kalinke et al., 2017). Numerous studies have demonstrated that biochar promotes growth by boosting the activity of plant antioxidant enzymes, which can strengthen plant defense systems (Li et al., 2015; Wang et al., 2014), yield, and beneficial soil features, all of which can ultimately enhance crop quality and nutritional value (Olmo et al., 2014). Biochar application is recognized as a cost-effective and sustainable practice for reducing TM mobility in polluted soils, limiting bioavailability by reducing adsorption and translocation of TMs (Zn, Cd, and Pb) from soil to plant aerial organs (Marchand et al., 2016; Ibrahim et al., 2022). Biochar types with a high cation exchange capacity (CEC), alkaline pH, and high capacity for carbon sequestration could be suitable materials for absorbing TMs from polluted soil (Pehlivan et al., 2023). The high surface of the material allows the absorbance of a substantial quantity of water and nutrients that can resist soil decomposition (Kim et al., 2015; Xu et al., 2012).

Compared to other plants, bamboo plants are an inexpensive resource to remove pollutants from the soil, water, and air. Bamboo's large biomass quickly transforms it into a plant with a high potential for phytoremediation (Bian et al., 2020). Bamboo biochar (BB) and bamboo charcoal are two bamboo products with a high capacity to remove and absorb nitrate–nitrogen, foam gases, and particularly TMs, from the environment (Ma et al., 2010). Bamboo biochar might be a great alternative for plants to absorb TMs owing to the substantial surface area and wide range of mesoporous and microporous structures (Ouyang et al., 2014). Therefore, to increase plant tolerance in polluted agricultural soils, such as those damaged by mining, it is required to identify diverse BB forms and assess the effects of varying BB concentrations on plant defense systems. We here hypothesized that BB could increase plant tolerance to TMs through a number of fundamental mechanisms against ROS, including free radicals such as hydroxyl radical (• OH) and superoxide anion (O^2•−) as well as non-radical molecules, such as H_2O_2, and singlet $^1O_2$ (Sharma et al., 2012), by increasing plant antioxidants and improving glyoxalase (Gly) system, enhancing the effectiveness of phytochelatins and reducing ion transfer to plant aerial organs by restricting ion mobility (Sharma et al., 2012).

Brassica juncea is a member of the Brassicaceae family with a strong phytoremediation capacity (may collect a substantial quantity of TMs on the root surface) as well as favorable traits such as being a hyperaccumulator and having rapid growth in TM contaminated soil. This species can be utilized in polluted regions for soil rehabilitation and oil and feed production (Fiaz et al., 2014). With the potential use of *Brassica juncea* as a phytoremediation plant, our study aims to decrease soil contamination by using an economical and low-cost material and evaluate the impact of various BB levels on the reduction of TMs in four soil-polluted mining areas (Wang et al., 2016). We also seek a better understanding of the mechanisms involved in applying BB to increase *Brassica juncea* tolerance to TMs stress. On the other hand, the use of medical stone in growth media is feasible due to its high cation exchange capacity (CEC) and high surface area. Therefore, based on our current understanding, this study is the first to compare BB with incorporating a specific compost combination (consisting of medicinal stone, chicken dung, and sawdust) concerning the limitations of TM bioavailability in the soils surrounding these four mining sites. Additionally, using BB to enhance the phytoremediation capacity of *Brassica juncea* in this region might be considered a novel aspect of this research.

2. Material and method

2.1. Materials and methods applied

Contaminated soil used in the experimental set-up was obtained from four mines, namely Sarcheshmeh (S), Chadormalu (C), Gol-Gohar (G), and Anguran (A). Mine and Smelter contaminated soils provided from (A) Sarcheshmeh (29°56′∼40′N, 55°52′∼20′E Kerman Province, Iran, which is the second-largest copper deposit worldwide containing substantial amounts of molybdenum, gold, and other rare metals (B); Chadormalu (32°19′∼03′N, 55°31′∼40′E Yazd Province, Iran, the primary iron ore concentrated in the Middle East, with a production of seven million tons of iron ore per year (C); Gol-Gohar (29°6′∼04′N, 55°19′∼23′E Sirjan County, Kerman Province, Iran, is one of the largest iron ore mine in Iran with total reserve of 1.135 billion tons) (D); Anguran (36°37′∼28′N, 47°24′∼20′E Sheykhlar, Mahneshan County, Zanjan Province, Iran, is one of the primary zinc producers in Iran) (Fig. 1). The main pollution sources in these four areas are mine tailing, mine wastewater, and atmospheric deposition.

The mine locations were selected based on preliminary studies undertaken by other researchers, which identified a significant issue of high toxicity in these specific areas of the country. The soil sampling for the pot experiments was conducted by sampling small segments (20 m^2) of soil with depths ranging from 0–20 cm around the mines, which compose one sample per site. Then, the samples were packaged in polyethylene bags and transferred to the lab. The soil samples were manually grounded, dried, and shaded at ambient temperature, then sieved through a 2 mm sieve to produce the final form of pot soil. Nanchang Company, Jiangsu, China, provided bamboo biochar and medical stones. We have used chicken manure and sawdust collected from local wood factories in Iran. For the compost, chicken manure, medical stone (2.5 %), and 2:1 dry-weight base sawdust were mixed in a composter (130 L PVC) for 60 days (Li et al., 2015; Wang et al., 2016). The final compost with different concentrations of BB and the soils sampled from the natural mining sites were used as plant growing mix designated in completely randomized pot experiments. The treatments consisted of five concentrations of biochar: A (0 % Control), B (2.5 % BB), C (5 % BB), D (7.5 % BB), and E (10 % BB) mixed in four types of contaminated soil Sarcheshmeh (S); Gol-Gohar (G); Chadormalu (C); Anguran (A) in four replicates. The pots were 15 × 12 × 9 cm^3 filled with 1 kg of growing media, including mining soil, compost (2.5 %), and BB. Ten *Brassica juncea* seeds (ShaanYou 16) for each

Fig. 1. The photos and the map show four polluted mining sites, including Sarcheshmeh (S) (A); Gol-Gohar (G) (B); Chadormalu (C) (C); Anguran (A) (D) four mining areas in which soil characteristics, and the assessment of toxic metal levels compost, and bamboo biochar was performed.

pot were sterilized in 3 % H_2O_2, then planted. Throughout the experiment, pots were kept in an open environment greenhouse for ten weeks. To analyze metabolic and biochemical parameters, the harvested *Brassica* samples comprising leaves and roots were transferred to the lab at the end of the experiments for the nutritional and physiological analysis and maintained in a Haier-China refrigerator at −20 °C.

The standard methods were used for the determination of soil characteristics such as electrical conductivity (EC), organic matter, and soil pH (1:2) (Li et al., 2015). We used ICP-AES to determine the total TMs in the natural mining soils based on the method of Hu et al., (2014). The DTPA/TEA extractable TMs (Cu, Pb, Cd, and Zn) were measured according to the protocol of Lu et al. (2014). Nitrogen sorption analysis at 77 K (TristarII 3020, Micromeritica Instrument Corporation, USA) was performed for the determination of BB surface area in a surface analyzer (Brunauer–Emmett–Teller (BET) according to the method of Mahar et al. (2016).

2.2. *Determination of soil enzymatic activities*

Soil enzyme activities were measured after harvesting *Brassica juncea*. The soil enzymes were determined by the following methods of Abujabhah et al. (2016) for β-glucosidase, Yang et al. (2016) for urease, and Xian et al. (2015) for alkaline phosphatase.

2.3. *Toxic metal accumulation in the leaves and roots of Brassica juncea*

Ten weeks after the onset of experiments, the TM accumulation analyses were performed using Karimi's method (Karimi et al., 2013) with a few modifications. The roots and shoots were washed and cleaned separately with deionized water, then dried in an oven at 110 °C for 5–8 hours. The tissues (0.25 g of *Brassica* root samples and 0.50 g of shoot samples) were mixed with HNO_3-$HClO_4$ (3:1) at 75 °C for 30 min. before being centrifuged at 10.000 g for 10 min. The concentration of TMs was measured by ICP-AES according to Chen et al. (2010)

2.4. *Calculation of tolerance index (T.I.) and metal immobilization (MI)*

Shoot and root tolerance indexes were calculated to determine the efficacy of BB on plant tolerance under TM stress. The below formula evaluated theBB impact onTM immobilization (ITM):

Shoot tolerance index (TI) = shoot dry weight (DW) of treatments (g) / shoot dry weight (DW) of control (g).

Root tolerance index (TI) = root dry weight (DW) of treatments (g) / root dry weight (DW) of control (g).

The percentage of TM immobilization (%) = DTPA extractable TMs in control - DTPA extractable TMs in the treatment / DTPA extractable TMs in control × 100

DTPA (diethylene triamine penta-acetic acid)

2.5. *Determination of plant antioxidant enzyme activities*

0.5 g of *Brassica juncea* leaves were ground with mortar and pestle, and the obtained powder was mixed with liquid nitrogen (LN). Then, 3 ml of pH 7.6 phosphate buffer was added to powdered leaves; then, the supernatant was obtained by centrifugation at 3000–4000 $\times g$ at 5 °C for 15 min.

According to the method used by Upadhyay et al. (2021), the superoxide dismutase (SOD) activity (EC 1.15.1.1) was assessed using photoreduction nitro blue tetrazolium (NBT) by measuring absorbance at 560 nm. The Liu technique (Liu et al., 2014) was used to determine the peroxidase activity (POD, EC 1.11.1.7) by wavelength absorption of 470 nm (0.001/min. at 25 °C). Using the extinction coefficient of H_2O_2 at 39.4 M^{-1} cm^{-1} at 240 nm, the catalase (CAT) (EC 1.11.1.6) activity was estimated (Azeem et al., 2023). According to the protocol used by Rezayian et al. (2023), phenylalanine ammonia-lyase (PAL) activity was also evaluated. For this, various concentrations of cinnamic acid were utilized, and PAL activity was measured at 290 nm for a duration of 25 min.

2.6. *Determination of glyoxalase (Gly) activity*

Using the technique described by Hasanuzzaman et al. (2011), the activity of glyoxalase (Gly I) was measured. The protein extract recorded an absorbance of 412 nm for 1 min. while the Gly II activity was monitored using the technique at 240 nm for 1 minute. According to the protocol used by Mohi-Ud-Din et al. (2021), methylglyoxal (MG) was further detected using a 200–500 nm absorbance range for 17 cycles by 1 min. intervals

2.7. *Glutathione, ascorbic acid, and phytochelatin content*

The GSH content data was obtained by subtracting the GSSG content from the total glutathione amount (Gill et al., 2015). AsA content has been estimated by the absorbance of supernatants at 265 nm via the AsA standard curve (Bhuyan et al., 2019). Phytochelatin concentration was obtained by non-protein thiol extraction and absorbance reading at 412 nm by the Ghorbani et al. (2022) method.

2.8. Determination of *Brassica juncea* chlorophyll pigments

Chlorophyll *a*, chlorophyll *b*, and carotenoids were calculated using protocol by Yusefi-Tanha et al., (2023) based on the absorbance rate in 663, 645, and 470 nm, respectively. Data were given as mg/g of fresh weight by the following formulas:

Chlorophyll $a = 12.25 \times$ (A value of 663 nm) $- 2.79 \times$ (A value of 647 nm).
Chlorophyll $b = 21.50 \times$ (A value of 647 nm) $- 5.10 \times$ (A value of 663 nm).
Carotenoid $= 1000 \times$ (A value of 470 nm) $- 1.82 \times$ (Chlorophyll a) $- 95.15 \times$ (Chlorophyll b) / 225.
Total Chlorophyll = Chlorophyll a + Chlorophyll b

2.9. Statistical analysis

Using the R software, a 2-way factorial design with four repeats was employed to analyze variance (ANOVA). The research was conducted using a single completely randomized design (CRD), and Tukey's honest significant difference (HSD) test was used to assess the mean differences between treatments at a probability level of $p < 0.05$.

3. Results

3.1. Soil characterization

The main characteristics of soils sampled from four naturally contaminated sites, including Sarcheshmeh (S), Chadormalu (C), Gol-Gohar (G), and Anguran (A), were measured along with total soil TMs (Cu, Pb, Zn, and Cd) (Table 1).

3.2. Bamboo biochar impact on soil EC and PH

Data analysis of the effect of BB on soil EC and pH revealed a significant distinction between different treatments ($P<0.001$), which demonstrated that applying BB significantly enhances soil structure while raising soil EC and pH values. The application of 10 % BB to the various polluted soils collected near the mines resulted in the highest increases in soil EC and pH values, with respective increases of 59 % and 11 % in Sarcheshmen, 93 %, and 10 % in Gol-Gohar, 80 % and 10 % in Chadormalu, and 72 % and 10 % in Anguran mining sites compared to control soils. Fig. 3 shows that the doses of 2.5 %, 5 %,7.5 %, and 10 % BB increased the pH and EC values of the soil.

3.3. Impact of bamboo biochar on the enzymatic activities in different natural mining soils

To evaluate the impact of BB on the soil enzymatic activities, β-glucosidase, urease, and alkaline phosphatase were measured. Four mine-polluted soils showed an increasingly large disparity in the soil enzyme activity after different treatments ($P<0.001$). The application of a 10 % BB dose to the soils of Chadormalu and Gol-Gohar resulted in the most significant increases in soil enzyme activity, with a 97 % rise in -glucosidase and 132 % and 97 % rise in urea and alkaline phosphatase activities, respectively, in compared to their controls. However, the impact of lower BB doses also revealed a significantly higher increase in soil enzymatic activities. 2.5 % BB increased -glucosidase, urease, and alkaline phosphatase activities in Gol-Gohar, Anguran, and Anguran soils by 16 %, 26 %, and 14 %, respectively, compared to the controls (Fig. 4). Results showed that BB positively impacted enzymatic activities in naturally mine-polluted soils.

Table 1
Significant characteristics of toxic metal contaminated soils of four mines; the data are given as mean (n=4).

Major soil characteristics	S-soil	C-soil	G-soil	A-soil
pH	8.02	7.77	7.89	8.09
Silt %	37.29	41.24	42.12	49.21
Clay %	1.77	1.65	1.11	1.63
Sand %	65.21	69.33	63.22	69.63
EC_e ($\mu S\ cm^{-1}$)	376	389	423	455
Total TMs in soil	**S-soil**	**C-soil**	**G-soil**	**A-soil**
Cu	8976	5234	4876	3356
Pb	2717	3567	2987	2821
Zn	6130	8530	8110	12670
Cd	415	713	523	610
Extractable TMs in soil	**S-soil**	**C-soil**	**G-soil**	**A-soil**
Cu	815	443	428	389
Pb	248	288	269	262
Zn	653	786	754	1194
Cd	41	68	48	54

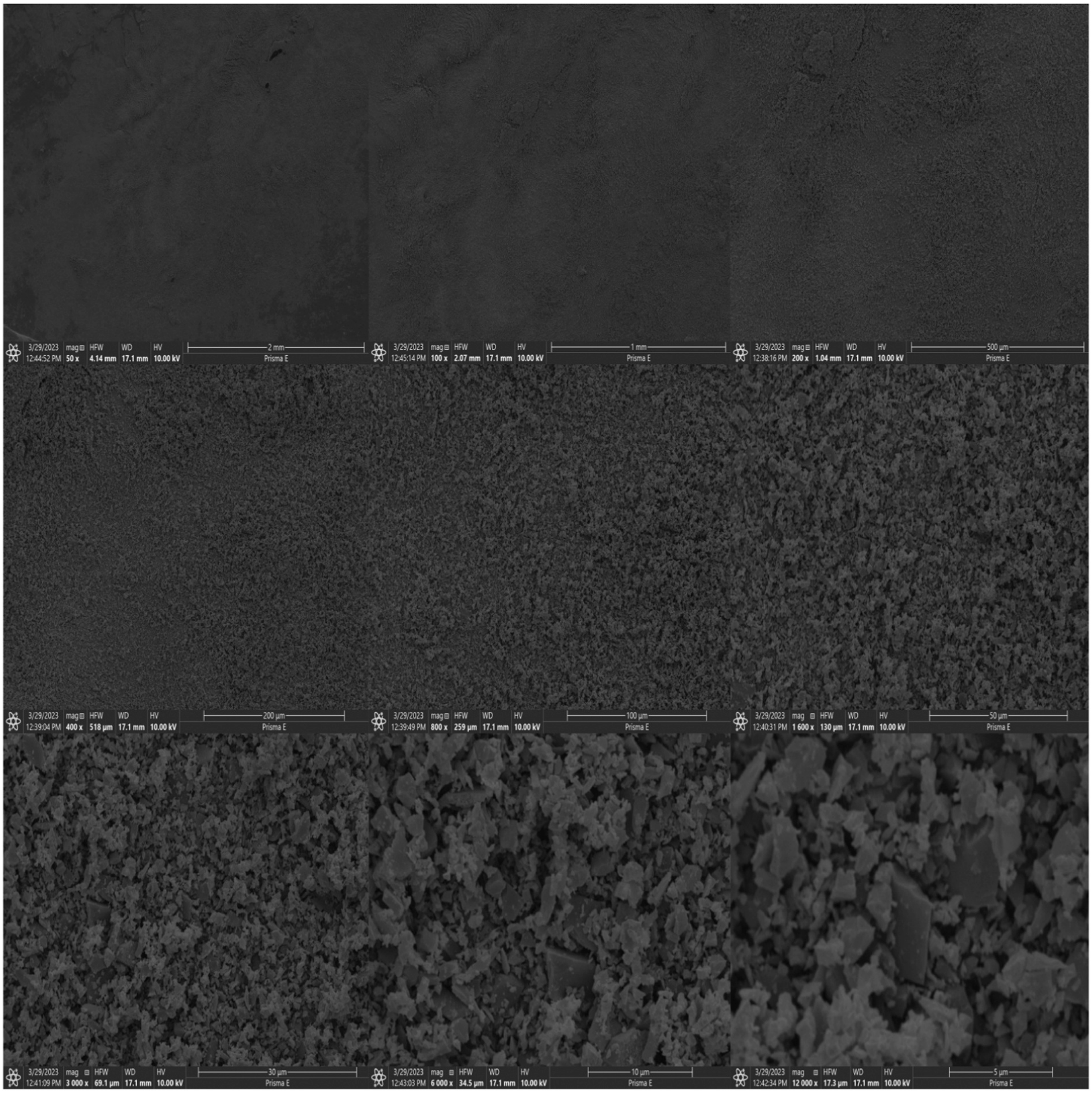

Fig. 2. Bamboo biochar visualized under a scanning electron microscope, which showed the surface area at high magnification.

3.4. Impact of bamboo biochar on TM bioavailability

The availability of TMs (Cu, Pb, Zn, and Cd) under BB conditions was monitored and analyzed. The results of TM extraction from soils showed considerable variation between different TM concentrations in various treatments for Sarcheshmeh (S), Gol-Gohar (G), Chadormalu (C), Anguran (A)($p<0.001$), which revealed that BB significantly reduced the TM availability in the soils (Fig. 5). The findings indicated that a dose of 10 % BB caused the most significant decrease in TM bioavailability. Sarcheshmeh (S) polluted soils showed a 71 %, 70 %, 82 %, and 63 % reduction in Zn, Pb, Cd, and Cu bioavailability relative to control. However, other polluted sites also significantly reduced TM bioavailability by 10 % BB and the DTPA-extractable TMs decreased in the following order: Cd (82 %) > Zn (71 %) > Pb (70 %) > Cu (63 %) in Sarcheshmeh (S) soil; Cd (70 %) > Pb (57 %) > Cu (47 %) > Zn (42 %) in Gol-Gohar (G) soil; Cd (75 %) > Pb (57 %) > Zn (51 %) > Cu (48 %) in Chadormalu (C) soil; Cd (72 %) > Zn (70 %)> Pb (59 %)> Cu (51 %) in Anguran (A) soil (Fig. 5).

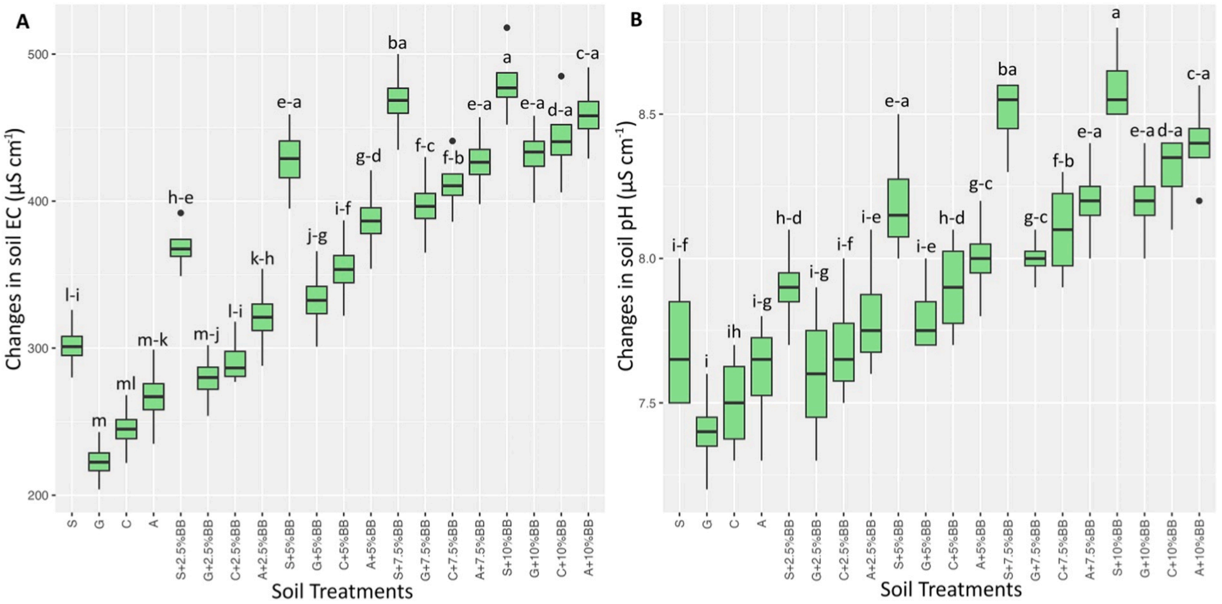

Fig. 3. The impact of different bamboo biochar ratios on soil structures (EC (A) and pH (B)). The boxes present the lower and upper quartiles (25–75 %). The whiskers show the min–max values. Lines in the boxes indicate median values (n=4). The treatments include four polluted mine soils (Sarcheshmeh (S); Gol-Gohar (G); Chadormalu (C); Anguran (A)) alone or in combination with five different concentrations of bamboo biochar (0 % control, 2.5 %, 5 %, 7.5 %, and 10 %). The lowercase letters (a, b, c, d, etc.) show significant differences among treatments based on Tukey′s HSD test ($p< 0.05$).

3.5. *Impact of bamboo biochar on TM translocation to plant shoots*

Brassica juncea shoots were tested for Cu, Pb, Zn, and Cd, and the data analysis revealed a substantial difference in the TM contents in different mine-polluted areas ($P<0.001$). A reduction trend in TM contents in shoots by BB application was obtained. The highest reduction of metal content in the *Brassica juncea* shoots grown in different mine soils mixed with BB was in Sarchemesh (S) with 57 %, 73 %, and 88 % reduction in Cu, Pb, and Cd, along with Anguran (A) (60 % reduction in Zn compared to control). This demonstrated that adding BB to soils reduced the quantity of bioavailable TMs and caused minimal translocation in the shoots of *Brassica* plants grown in mining soil. However, 10 % BB application to the Sarchemesh (S), Gol-Gohar (G), Chadormalu (C), and Anguran (A) soils had the most prominent advantage from BB treatment in terms of a decrease in TM transfer to bamboo shoots by 57 %, 46 %, 48 %, and 53 % reduction in Cu, 73 %, 58 %, 61 %, and 66 % reduction in Pb, 54 %, 33 %, 40 %, and 60 % reductions in Zn, and 88 %, 61 %, 75 %, and 71 % reduction in Cd in compare with control treatments respectively (Suppl. Fig. 1.).

3.6. *Impact of bamboo biochar on TM accumulation in Brassica juncea roots*

The results demonstrated that using BB prevented TM accumulation in plant roots, which showed the same restriction pattern in the shoot TM concentrations. We discovered a significant barrier that prevented shoot accumulation when different BB BB ratios were supplied ($p<0.001$). The data confirms that TM uptake in roots significantly influenced by the high adsorption capacity of applied BB, which can fix TMs in the mine soils and lead to immobilization. The 10 % BB in the mine soils resulted in the most significant reduction of TMs (Cu, Pb, Zn, and Cd) by 70 %, 71 %, 58 %, and 89 %, respectively, in Sarchemesh soils (S), 50 %, 66 %, 40 %, and 62 % in Gol-Gohar soils (G), 53 % in Chadormalu (C), and 55 % in Anguran soils (A) when compared to their control treatments (Suppl. Fig. 2.).

3.7. *Impact of bamboo biochar on tolerance index (TI) and metal immobilization (MI)*

The data obtained by calculating TI in shoot and root indicated a significant difference among treatments ($P<0.001$), showing an increasing trend in TI with BB application. The highest percentage of TI in shoot and root of *Brassica* plants was obtained in the 10 % BB dose supplemented by 86 % and 40 % in Sarcheshmeh (S); 74 %, and 82 % in Gol-Gohar (G); 69 %, and 67 % in Chadormalu (C); and 71 %, and 56 % in Anguran (A) in comparison with their controls, respectively. On the other hand, calculations of the MI percentage in Cu, Pb, Zn, and Cd treatments displayed a significant increase in metal immobilization rate with different amounts of BB ($P<0.001$), in which 10 % BB resulted in the highest percentage of MI. Sarcheshmeh (S) polluted soils showed a 63 % reduction in Cu, 69 % in Pb, 54 % in Zn, and 82 % reduction in Cd, while Gol-Gohar contaminated soils showed a 43 % reduction in Cu, 57 % in Pb, 42 % in Zn, and 70 % reduction in Cd. Furthermore, Chadormalu (C) polluted soils represented a 47 % reduction in Cu, 57 % in Pb, 51 % in Zn, and 74 % in Cd, and Anguran (A) polluted soils showed a 51 % reduction in Cu, 59 % in Pb, 70 % in Zn, and 72 % in Cd compared to their

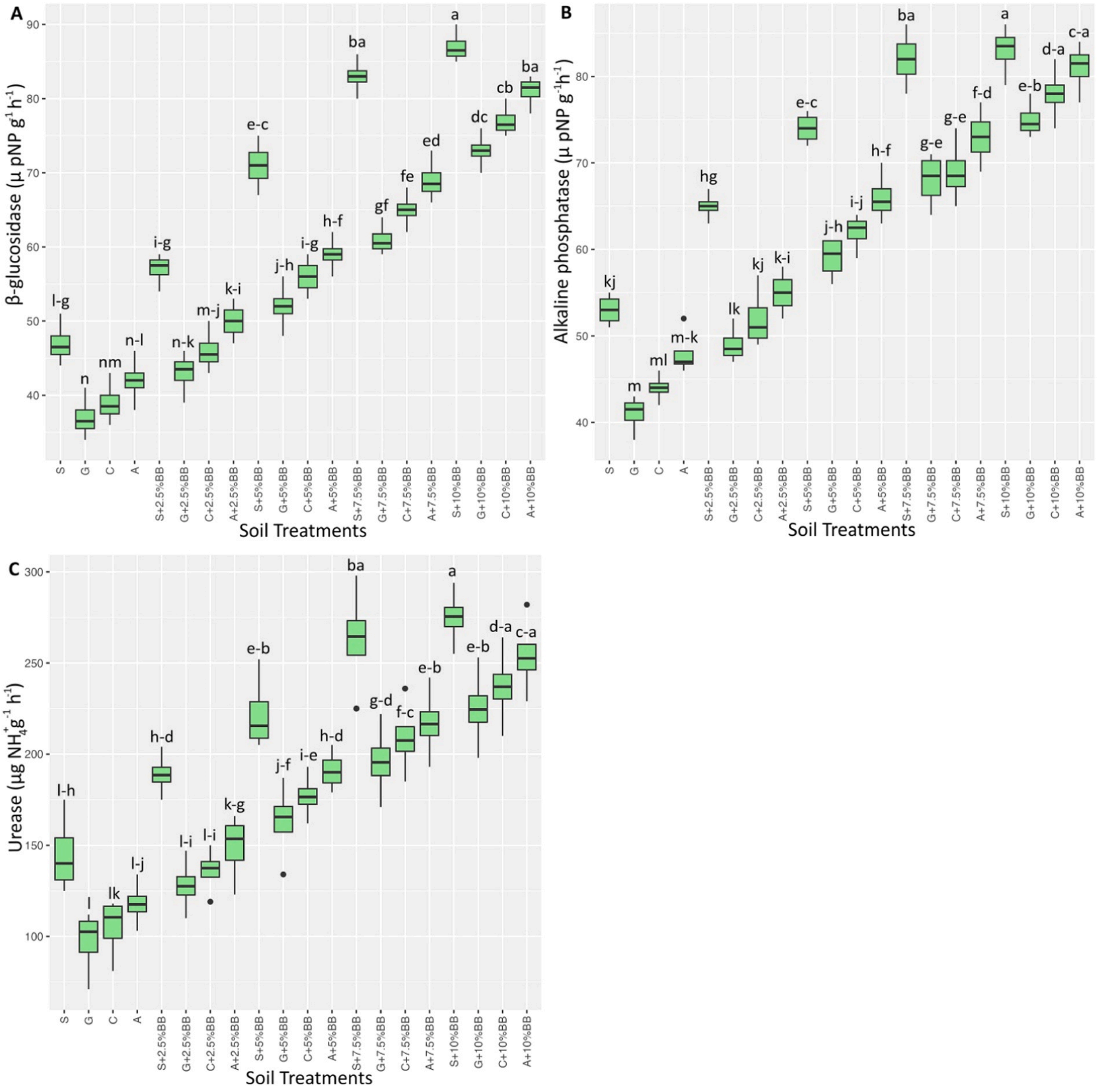

Fig. 4. The impact of different bamboo biochar ratios on soil enzymatic activities in Brassica (β-glucosidase (A), alkaline phosphatase (B), and urease (B)). The boxes present the lower and upper quartiles (25–75 %). The whiskers show the min–max values. Lines in the boxes indicate median values (n=4). The treatments include four polluted mine soils (Sarcheshmeh (S); Gol-Gohar (G); Chadormalu (C); Anguran (A)) alone or in combination with five different concentrations of bamboo biochar (0 % control, 2.5 %, 5 %, 7.5 %, and 10 %). The lowercase letters (a, b, c, d, etc.) show significant differences among treatments based on Tukey's HSD test ($p < 0.05$).

respective controls (Table 2).

3.8. *Impact of bamboo biochar on chlorophyll content of Brassica juncea*

Table 3 displays the carotenoid and chlorophyll levels of plants grown in natural mine-polluted soils supplemented with BB. The carotenoid and chlorophyll pigment amounts varied significantly between treatments ($p<0.001$). The most considerable improvement was related to the dose of 10 % BB in the soils, with a 34 % increase in chlorophyll-a in Chadormalu and Anguran soil, a 175 % increase in chlorophyll-b, 64 % increase in total Chlorophyll, and a 174 % increase in carotenoid content in Gol-Gohar soil, respectively, in comparison to their controls. This confirmed that BB increased photosynthetic pigment intactness in polluted sites.

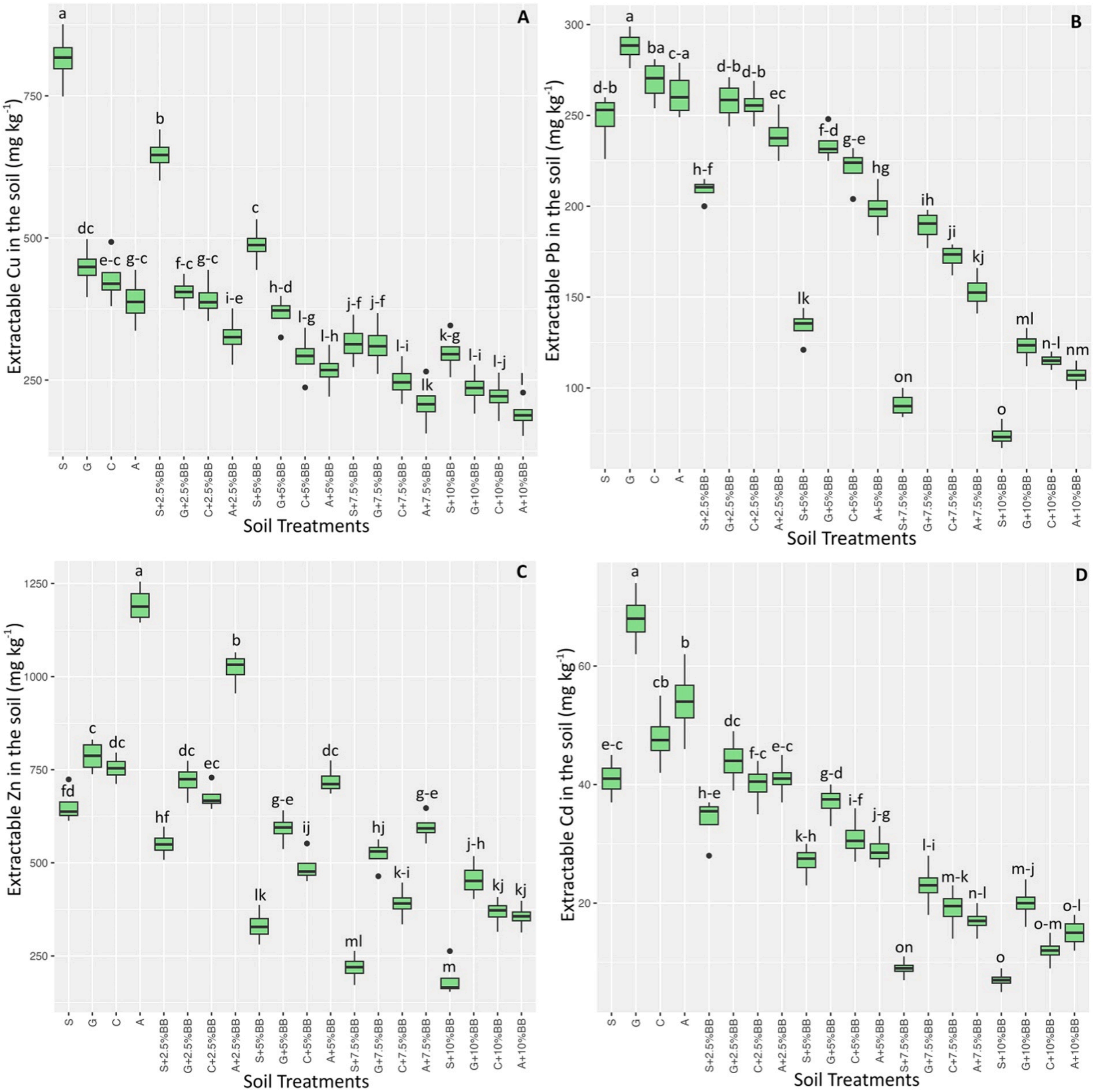

Fig. 5. The impact of different bamboo biochar ratios on extractable metal in the soil (Cu (A), Pb (B), Zn (C), Cd (D)). The boxes present the lower and upper quartiles (25–75 %). The whiskers show the min–max values. Lines in the boxes indicate median values (n=4). The treatments include four polluted mine soils (Sarcheshmeh (S); Gol-Gohar (G); Chadormalu (C); Anguran (A)) alone or in combination with five different concentrations of bamboo biochar (0 % control, 2.5 %, 5 %, 7.5 %, and 10 %). The lowercase letters (a, b, c, d, etc.) show significant differences among treatments based on Tukey's HSD test ($p < 0.05$).

3.9. *The impact of bamboo biochar on Brassica juncea root and shoot dry biomass*

The data analyses showed that the BB application significantly increased shoot and root dry biomass in *Brassica* plants grown in TM-contaminated soils of four mines ($p<0.001$), which revealed that BB application increases biomass in *Brassica*. The most influential ratio in increasing root and shoot dry biomass was the 10 % BB in four mining soils by 40 % and 85 % increase in Sarcheshme, 82 %, 73 % increase in Gol-Gohar, 67 %, 69 % increase in Chadormalu, 57 %, and 71 % increase in Anguran in compare with the controls, respectively. The results demonstrated that in four mine-polluted soils, plant biomass measured as the dry weight of roots and shoots could increase at all BB ratios. This revealed that introducing 2.5 % BB into the polluted mine soils resulted in the lowest increase in plant root and shoot biomass, by 16 % and 19 % in Sarcheshme, 25 % and 12 % in Gol-Gohar, 21 % and 6 % in Chadormalu, and 21 % and 7 % in Anguran, respectively, in comparison to control (Suppl. Fig. 3.).

Table 2

Changes in the tolerance index (TI) of the Brassica shoot, root, and metal immobilization (MI) in four polluted mine soils supplemented with 0 % control, 2.5 %, 5 %, 7.5 %, and 10 % bamboo biochar relative to control. The data display the mean ± standard error of four replicates.

Treatments	TI(shoot)	TI(root)	MI(Cu)	MI(Pb)	MI(Zn)	MI(Cd)
S	1± 0.05^{a}	1±0.04^{a}	nd	nd	nd	nd
G	1± 0.05^{a}	1 ±0.04^{a}	nd	nd	nd	nd
C	1± 0.05^{a}	1± 0.04^{a}	nd	nd	nd	nd
A	1± 0.05^{a}	1±0.04^{a}	nd	nd	nd	nd
S +2.5 % BB	1.19±0.05$^{i\text{-}f}$	1.17±0.04^{ih}	20.61±4.24 $^{h\text{-}d}$	15.48±2.48 $^{g\text{-}e}$	15.01±4.48 $^{g\text{-}d}$	17.18± 3.71^{fe}
G +2.5 % BB	1.12±0.05$^{i\text{-}g}$	1.25±0.04 $^{h\text{-}f}$	9.40±4.24 ^{hg}	10.25±2.48 $^{h\text{-}f}$	7.93±4.48^{gf}	34.71±3.71^{ed}
C +2.5 % BB	1.06±0.05^{ih}	1.21±0.04$^{i\text{-}g}$	7.03±4.24 ^{h}	4.56±2.48 ^{hg}	10.21±4.48 $^{g\text{-}e}$	15.58±3.71^{fe}
A +2.5 % BB	1.07±0.05^{ih}	1.21±0.04$^{i\text{-}g}$	15.75±4.24 $^{h\text{-}f}$	8.44±2.48 $^{h\text{-}f}$	14.48±4.48 $^{g\text{-}d}$	23.40±3.71^{e}
S +5 % BB	1.41±0.05 $^{g\text{-}d}$	1.28±0.04 $^{h\text{-}e}$	40.05±4.24$^{e\text{-}b}$	45.68±2.48^{cb}	48.77±4.48^{ba}	34.24± 3.71^{ed}
G +5 % BB	1.3±0.05 $^{h\text{-}e}$	1.52±0.04$^{e\text{-}b}$	17.88±4.24 $^{h\text{-}e}$	18.59±2.48 ^{fe}	24.71±4.48$^{f\text{-}c}$	45.13±3.71^{dc}
C+ 5 % BB	1.26 ± 0.05$^{i\text{-}e}$	1.43 ± 0.04 $^{g\text{-}c}$	32.19 ± 4.24 $^{f\text{-}c}$	17.85 ± 2.48^{fe}	35.22 ± 4.48$^{d\text{-}b}$	34.18 ± 3.71^{ed}
A+5 % BB	1.30±0.05 $^{h\text{-}e}$	1.40±0.04 $^{h\text{-}d}$	31.49±4.24 $^{g\text{-}c}$	23.79±2.48^{ed}	39.61±4.48^{cb}	46.40±3.71^{dc}
S +7.5 % BB	1.73±0.05$^{c\text{-}a}$	1.37±0.04 $^{h\text{-}d}$	60.87±4.24^{ba}	63.30±2.48^{a}	66.03±4.48^{a}	77.69±3.71^{ba}
G +7.5 %BB	1.51± 0.05$^{e\text{-}b}$	1.70± 0.04^{ba}	30.24± 4.24 $^{g\text{-}c}$	34.30± 2.48^{dc}	33.56± 4.48$^{e\text{-}b}$	66.24± 3.71^{ba}
C +7.5 % BB	1.45± 0.05 $^{f\text{-}c}$	1.57± 0.04$^{d\text{-}b}$	41.21± 4.24$^{d\text{-}a}$	36.05± 2.48^{dc}	48.26± 4.48^{ba}	59.70± 3.71^{cb}
A+7.5 % BB	1.45± 0.05 $^{f\text{-}b}$	1.46±0.04 $^{f\text{-}b}$	44.76±4.24$^{c\text{-}a}$	41.23±2.48^{c}	49.92±4.48^{ba}	66.9±3.71^{ba}
S +10 % BB	1.85±0.05^{a}	1.40±0.04 $^{h\text{-}d}$	63.44±4.24^{a}	69.94 ±2.48^{a}	54.03±4.48^{ba}	82.59±3.71^{a}
G +10 % BB	1.74± 0.05^{ba}	1.82± 0.04^{a}	43.44± 4.24$^{c\text{-}a}$	57.18± 2.48^{ba}	42.06± 4.48^{cb}	70.72± 3.71^{ba}
C +10 % BB	1.69± 0.05$^{d\text{-}a}$	1.67± 0.04$^{c\text{-}a}$	47.65± 4.24$^{c\text{-}a}$	57.13± 2.48^{ba}	51.41± 4.48^{ba}	74.34± 3.71^{ba}
A+ 10 % BB	1.71± 0.05$^{c\text{-}a}$	1.56±0.04$^{d\text{-}b}$	51.20±4.24$^{c\text{-}a}$	59.16±2.48^{a}	70.22±4.48^{a}	72.19±3.71^{ba}

Table 3

The effect of different levels of bamboo biochar on photosynthetic pigments (Chl-a, Chl-b, Total Chl, and Carotenoid) of Brassica. The data show the mean ± standard error of four replicates. The treatments include four polluted mine soils (Sarcheshmeh (S); Gol-Gohar (G); Chadormalu (C); Anguran (A)) alone or in combination with five different concentrations of bamboo biochar (%0 control), 2.5 %, 5 %, 7.5 %, and 10 %). The lowercase letters (a, b, c, d, etc.) show significant differences between treatments determined by Tukey's test ($p< 0.05$).

Treatment	Chl-a (mg g^{-1} F.w.)	Chl-b (mg g^{-1} F.w.)	Chl a+b (mg g^{-1} F.w.)	Caratenoids (mg g^{-1} F.w.)
S	6.02± 0.06 $^{l\text{-}j}$	1.98 ± 0.04 ^{l}	8 ± 0.09 ^{nm}	1.19 ± 0.09 $^{m\text{-}j}$
G	5.35 ± 0.06°	1.32 ± 0.04 ^{n}	6.61± 0.09^{q}	0.87 ± 0.09 ^{m}
C	5.48 ± 0.06^{on}	1.48 ± 0.04 ^{n}	6.96± 0.09^{qp}	0.92 ± 0.09 ^{m}
A	5.65± 0.06$^{o\text{-}m}$	1.72± 0.04 ^{m}	7.37± 0.09^{po}	0.98 ± 0.09 ^{ml}
S +2.5 % BB	6.51± 0.06^{ih}	2.68 ± 0.04^{i}	9.19± 0.09^{ji}	1.71 ± 0.09$^{j\text{-}g}$
G +2.5 % BB	5.79 ± 0.06 $^{n\text{-}l}$	1.75 ± 0.04 ^{m}	7.54± 0.09^{on}	1.02 ± 0.09 ^{ml}
C+2.5 % BB	5.92± 0.06 $^{m\text{-}k}$	1.87 ± 0.04 ^{ml}	7.79± 0.09$^{o\text{-}m}$	1.09± 0.09 $^{m\text{-}k}$
A +2.5 % BB	6.18± 0.06$^{k\text{-}i}$	2.08 ± 0.04^{lk}	8.26 ± 0.09 ^{ml}	1.31 ± 0.09 $^{m\text{-}j}$
S + 5 % BB	7.12 ± 0.06 $^{f\text{-}d}$	3.43 ± 0.04^{fe}	10.55± 0.09 ^{fe}	2.28 ± 0.09$^{f\text{-}e}$
G+ 5 % BB	6.27± 0.06^{ji}	2.23 ± 0.04^{k}	8.5± 0.09 ^{lk}	1.45 ± 0.09$^{l\text{-}i}$
C +5 % BB	6.39± 0.06^{i}	2.45 ± 0.04^{j}	8.84± 0.09^{kj}	1.58 ± 0.09$^{k\text{-}h}$
A +5 % BB	6.75± 0.06 ^{hg}	2.78 ± 0.04^{i}	9.53± 0.09^{ih}	1.85 ± 0.09$^{i\text{-}f}$
S +7.5 % BB	7.81± 0.06^{ba}	4.12 ± 0.04^{b}	11.93± 0.09^{b}	2.69 ± 0.09$^{b\text{-}a}$
G +7.5 % BB	6.79± 0.06 $^{h\text{-}f}$	2.89 ± 0.04^{ih}	9.68± 0.09 ^{h}	1.94 ± 0.09$^{i\text{-}e}$
C +7.5 % BB	6.88 ± 0.06 $^{g\text{-}e}$	3.09 ± 0.04 ^{hg}	9.99± 0.09 ^{hg}	2.04 ± 0.09 $^{h\text{-}d}$
A + 7.5 % BB	6.98± 0.06 $^{g\text{-}e}$	3.22 ± 0.04^{gf}	10.2± 0.09 ^{gf}	2.15 ± 0.09$^{g\text{-}c}$
S + 10 % BB	8.02± 0.06^{a}	4.41 ± 0.04^{a}	12.43± 0.09^{a}	2.88 ± 0.09^{a}
G + 10 % BB	7.19± 0.06^{ed}	3.64 ± 0.04^{ed}	10.83± 0.09^{ed}	2.39 ± 0.09$^{e\text{-}a}$
C+ 10 % BB	7.39± 0.06^{dc}	3.76 ± 0.04^{dc}	11.15± 0.09^{dc}	2.51 ± 0.09$^{d\text{-}a}$
A+ 10 % BB	7.62± 0.06^{cb}	3.97 ± 0.04^{cb}	11.59± 0.09^{cb}	2.63 ± 0.09$^{c\text{-}a}$

3.10. Impact of bamboo biochar on phytochelatin, ascorbic acid, and GSH content of Brassica juncea

Phytochelatin, ascorbic acid, and GSH content measurements indicated significant variations between BB ratios in mine-polluted soils ($p<0.001$). The content of these three indexes was found immoderate in all four polluted soils, including Sarcheshmeh, Gol-Gohar, Chadomalu, and Anguran, in comparison to their control treatments, with increments in phytochelatin content by 102 %, 180 %, 148 %, and 125 %, ascorbic acid by 68 %, 138 %, 117 %, and 99 %, and GSH content by 76 %, 107 %, 96 %, and 88 %, respectively. On the other hand, the findings showed that phytochelatin, ascorbic acid, and GSH contents were potentially raised in all BB concentrations. However, the decrease in all three indices was maximum for the low BB concentrations. Therefore, applying 2.5 % bamboo biochar to the Sarcheshmeh and Anguran polluted soils led to the weakest improvements in phytochelatin, ascorbic acid, and GSH contents, with respective reductions of 25 %, 19 %, and 18 % compared to their control treatments (Suppl. Fig. 4.).

3.11. *Impact of bamboo biochar on plant antioxidant machinery and glyoxalase activity of Brassica juncea*

Antioxidant enzyme activity tests revealed a significant difference between diverse natural soils supplemented with varying amounts of BB ($p<0.001$), demonstrating an upward trend in antioxidant activity. Plant treatments exposed to four mine-polluted soils (Sarcheshmeh, Gol-Gohar, Chadormalu, and Anguran) supplemented with 10 % BB increased SOD activity by 65 %, 81 %, 74 %, and 73 %, POD activity by 75 %, 147 %, 125 %, and 104 %, CAT activity by 55 %, 73 %, 68 %, and 63 %, and PAL activity by 104 %, 75 %, 160 %, and 145 %. Glyoxalase activity (GLY1 and GLY2) throughout treatments in various mine-polluted soils, on the other hand, developed significant variance between treatments ($p<0.001$). This has demonstrated that adding BB into contaminated soils promotes glyoxalase activity. According to the findings, the plants exposed to Gol-Gohar soil under 10 % BB showed the most significant increase in GLY1 and GLY2 compared to control treatments, with 158 % and 135 % values, respectively. This ultimately lowers the amount of MG in the plants (Suppl. Fig. 5.).

4. Discussion

Biochars can improve pH, cation exchange, water-holding capacity (WHC), organic carbon concentrations, electrical conductivity (EC), and TM bioavailability in the soil (Calcan et al., 2022) in addition to improving soil aggregation porosity and stability, nutrient cycling, penetration resistance, and tensile strength (Pehlivan and Wang, 2022). Biochars can also lower soil acidity, which might aid in improving soil fertility (Shetty and Prakash, 2020). Given that these materials can release cations into the soil after being added, the pH and EC of the soil gradually rise (Kim et al., 2015; Al-Wabel et al., 2015). Thus, this enhances plant growth and development while reducing the bioavailability of TMs (Calcan et al., 2022). The bioavailability and mobility of TMs can be limited when biochar converts the accessible portion into a geochemically stable fraction (Kim et al., 2015; Al-Wabel et al., 2015; Fellet et al., 2011).

Pyrolysis of feedstock such as bamboo enhances the soil pH, which is associated with the release of alkali salts in soils (Lu et al., 2014; Houben et al., 2013). In accordance with that, BB showed an alkaline character. Data revealed that applying different percentages of BB increased soil properties (soil pH and soil EC), which was in line with earlier studies (Al-Wabel et al., 2015; Shen., et al., 2016; Zhang et al., 2019).

For monitoring TM contamination, as well as agricultural practices and soil management, soil enzymatic activity is a crucial indication of soil health (Yang et al., 2016). Consequently, measuring the activity of soil enzymes can serve as a baseline for documenting soil pollution with TMs in a specific location. For instance, β-glucosidase releases low molecular weight sugars that give energy to soil bacteria (Pathan et al., 2017), and our results indicated that BB could boost β-glucosidase activity in the soil. Also, alkaline phosphatase stimulates mineralization in soil, thus enhancing root growth. Biochar application might have strengthened such mineralization in our work since BB and compost contain phosphorus (Lehmann et al., 2011). However, ureases may accelerate the soil's ability to process organic materials and nutrients. According to reports, BB helps soil microbes regulate nutrient transformations and cycling; hence, BB could have enhanced plant soil by stimulating urease enzymes (Lehmann et al., 2011). Our data show that BB's presence significantly elevated the soil's enzymatic activity, specifically alkaline phosphatase, β-glucosidase, and ureases. This may be connected to BB's function in reserving essential nutrients and carbon in the soil (Kotroczó et al., 2014). The role of BB in increasing alkaline phosphatase, β-glucosidase, and urease activities in TM-contaminated soils has previously been reported (Abujabhah et al., 2016; Mackie et al., 2015). Also, alkaline phosphatase-glucosidase and urease activities in polluted mine soils were reported to increase with the integration of BB (Ali et al., 2017), consistent with our findings. Therefore, we proposed that BB would be a suitable choice to improve soil enzymatic activity, soil characteristics, and soil amendation, leading to TM immobilization and limited availability in plant absorption and translocation.

Toxic metals with a high probability of migrating from the soil to plants are referred to as DTPA extractable TMs, which, in soils, can be immobilized and retained by biochar materials. This proceeds through a variety of mechanisms, including TM absorption, the exchange of ions between metal cations and protons released from the specific biochar, the assembly of phosphate chelates during precipitation, and particular complexes with other organic matter (Lu et al., 2017; Yang et al., 2016). As a result of the ability of biochar to form a complex with the dissolved organic HCO_3 and C, ion immobilization in soil is enhanced, translocation to plants diminishes, and TM concentration in soil lowers since TMs are adsorbed on the surface of biochar (Shen et al., 2016; Lucchini et al., 2014). Our findings showed that the treatments with DTPA extractable TMs, including Cu, Pb, Zn, and Cd, decreased with BB application in the growing media compared to controls. It has also been reported that biochar improving ecophysiological parameters in plants reduces oxidative stress and protects plant cells from damage caused by abiotic stress such as heavy metal toxicity (Hasnain et al., 2023). We suggest that bamboo's high porosity is supposed to be critical in absorbing metal ions and forming complexes. -COOH, -OH, and $C{=}N$ in BB, could also play a decisive role in metal ions' adsorption (Li et al., 2015; Lu et al., 2017). According to reports (Ahmad et al., 2016; Marchand et al., 2016), BB could increase dissolved organic matter (DOM) in polluted soils, creating complex forms with TMs like Cu and Pb. Additionally, the effects of biochar on DTPA-extractable TMs were reported upon BB application (Ali et al., 2017), orchard prunes biochar (Fellet et al., 2011), and rice straw biochar (Yang et al., 2016), which demonstrated that these substances lower the concentrations of bioavailable TMs. Moreover, it can restrict the movement of ions from the plant's roots, drive them to plant aerial organs, and mitigate phytotoxicity in plants. Our data showed that BB significantly reduced phytoextraction indices, including translocation factor and bioaccumulation factor in plant shoots and roots, which is related to the ability of BB to immobilization TMs in the soil and might be one of the significant reasons for improved plant tolerance grown in natural mine-polluted soils. Bamboo biochar can immobilize TMs in the soil through cation exchange, complexation, reduction and precipitation, and electrostatic interactions, which can accumulate metals in the root surface and limit the translocation from root to shoot (Ghandali et al., 2024).

Toxic metals in plants lead to the generation of ROS compounds, including H_2O_2, $O_2\bullet-$, and MG MG and OH OH groups, which induce oxidative stress, disrupt permeability in cell membranes, damage cell molecules` structures, and finally lead to cell death. However, plants have intrinsic defense mechanisms such as antioxidants and Glyoxalases (Gly), which can scavenge ROS and MG (Hasanuzzaman et al., 2011). In addition, ROS compounds destroyvital molecules and damage the cell compartment. In this situation, the activation of SOD, GPX, and CAT as a primary antioxidant activity are plant mechanisms to react with these damaging molecules, which can transform them into other molecules, including water and oxygen. The presence of TMs in mine-polluted soils decreases the antioxidant enzyme activity of SOD, POD, CAT, and PAL (Hasanuzzaman et al., 2011). *Brassica juncea*'s antioxidant enzyme activities were significantly raised in our work by adding BB in various doses. Glyoxalase (Gly) activity revealed comparable findings, demonstrating that BB treatment boosted Glyoxalase (Gly) I and Glyoxalase (Gly) II, resulting to a decrease of ROS and MG in plants under TM toxicity, which can be an effective mechanism in TM detoxification in plants (Pehlivan et al., 2021). *Zea mays* L. (Zha et al., 2022) and *Brassica juncea* (Ali et al., 2017) have also been reported on BB's effect on enhancing antioxidant activity.

Glutathione (GSH) is a thiol molecule that plays an integral part in raising plant tolerance to stress. Consequently, it is crucial in augmenting plants' adaptation to cellular stress, influencing various biochemical processes (Vezza et al., 2019). GSH acts as a precursor of phytochelatins (PCs) and is an antioxidant in plant cells under TM toxicity (Jung et al., 2019). Phytochelatin (PC) synthase, a self-regulatory and inactivating enzyme, is triggered in plants whenever metal (loid)s start to exceed toxic levels for the cell (Li et al., 2020). It works by binding metal (loid)s with peptide groups and may be crucial for plants' ability to absorb metal ions during TM toxicity (Ogawa et al., 2011).

On the other hand, the efficient redox system of ascorbic acid, a natural antioxidant, can preserve plant cells under the ROS effect by oxidizing them into dehydroascorbic acid (Bilska et al., 2019). Also, ascorbic acid can increase photochemical activity, improving plant photosynthesis (Chen et al., 2021). Here, the application of BB considerably enhanced Glutathione (GSH), Phytochelatin (), and Ascorbate acid indices in *Brassica juncea* in TM-contaminated natural soils obtained from various mines, according to our findings. Therefore, we propose that using BB can assist in reducing TM toxicity by promoting the chelate formation of PCs and GSH with metal ions and scavenging ROS with rising ascorbic acid concentrations. Numerous research has discussed the significance of, ASA, and GSH indicators in enhancing plant tolerance to TMs stress, and the data in the current study are consistent with these reports (Souri et al., 2020; Aborode et al., 2016; Alamri et al., 2018). According to literature (Xian et al., 2015; Hmid et al., 2015), TM-derived ROS production can damage cell walls and hinder the development of carotene and chlorophyll pigments, which in turn causes plant leaves to chlorosis. In Brassica leaves, it also causes disruptions in the absorption of Fe and Mg (Cenkci et al., 2010). Our findings indicated that while the toxicity of TMs in mine soils limits the number of pigments involved in photosynthesis, increasing levels of BB significantly improved the content of carotenoids and chlorophyll a and b in *Brassica juncea* leaves harvested after grown in mine-polluted soils. This ability of BB might have been acquired by the release of P and N from biochar, which can accelerate the biosynthesis of chlorophyll a, b, and other protective pigments in the photosystem as carotenoids (Fiaz et al., 2014). The impact might be explained by the ROS components' potential to be scavenged being initiated by BB's activation of antioxidant functions (Wang et al., 2014). Accordingly, different biochar types have increased chlorophyll pigment intactness and photosynthetic efficiency in *Malus hupehensis* Rehd and *Brassica juncea* leaves (Wang et al., 2014; Ali et al., 2017). Wang et al. (2014) also reported that adding biochar to soil promotes plant growth and yield and increases photosynthetic rate, which is consistent with our study's findings. According to the data, the use ofBB boosted plant biomass and plant growth indices in treatments for Brassica, including root dry weight, shoot dry weight, and shoot length under contaminated soils of mines. Several studies have confirmed the beneficial effects of biochar on plant biomass and the dry weight of roots, stems, and leaves (Houben et al., 2013; Shen et al., 2016; Al-Wabel et al., 2015). Consequently, based on our hypothesis, BB may contribute to an increase in plant biomass by enhancing antioxidant activity in plants and decreasing ROS-induced oxidative stress (Suppl. Fig. 6.).

5. Conclusion

Through growing *Brassica* plants in the mine-polluted soil of all regions, BB has demonstrated a high potential to be used as an efficient amendment in the soil and a helpful amelioration agent for reducing TM-caused plant oxidative stress. Our findings indicated that BB enhanced enzymes in the natural mining soil and altered soil physicochemical parameters, including pH and EC, reducing TM bioavailability and constraining the migration of ions to *Brassica* plants' aerial organs. Additionally, BB lowered soil acidity while promoting soil fertility and enhancing plant development. One of the essential processes for stimulating further plant TM tolerance is BB's ability to adsorb TMs, which lowers accumulation and translocation from roots to shoots. The findings demonstrated that BB treatment improved antioxidant enzyme activity, the glyoxalase system, GSH, PC, and AsA levels in *Brassica,* which mitigated plant oxidative stress by suppressing ROS and MG.

Additionally, BB helped with plant growth, biomass, and photosynthetic pigments. The findings demonstrated that 10 % BB was the most effective in restoring soil and increasing the durability of plants in mining soil. Therefore, *Brassica* plants might be suitable candidates for phytoremediation/preventing environmental contamination when mixed with BB, which might increase plant tolerance in these four mining zones. However, extra research is required to identify the most effective method of detoxifying the environment through plant phytoremediation using biochar via either physical or chemical activation. It is advisable to prioritize utilizing eco-friendly and cost-efficient organic materials, such as biochar activated with a range of nutrients and/or beneficial bacteria. This approach aims to mitigate soil toxicity caused by metals in different regions.

Funding

This work recieved financial support from the National Key Research and Development Program of China (2023YFD220120302).

CRediT authorship contribution statement

Yang Li: Writing – original draft, Software, Resources, Methodology, Formal analysis, Conceptualization. **Meisam Zargar:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Guohua Liu:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Abolghassem Emamverdian:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Abazar Ghorbani:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Necla Pehlivan:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Abolgassem Emamverdian reports financial support was provided by Nanjing Forestry University.

Data availability

Data will be made available on request.

Acknowledgments

We would like to extend our sincere gratitude and appreciation to Peijian Shi, Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, Jiangsu, China, for helping in the statistical analysis of the manuscript. This work was supported by RUDN University Strategic Academic Leadership Program.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103753.

References

Aborode, F.A., Raab, A., Voigt, M., Costa, L.M., Krupp, E.M., Feldmann, J., 2016. The importance of glutathione and phytochelatins on the selenite and arsenate detoxification in *Arabidopsis thaliana*. J. Environ. Sci. 49, 150–161. https://doi.org/10.1016/j.jes.2016.08.009.

Abujabhah, I.S., Bound, S.A., Doyle, R., Bowman, J.P., 2016. Effects of biochar and compost amendments on soil physico-chemical properties and the total community within a temperate agricultural soil. Appl. Soil Ecol. 98, 243–253. https://doi.org/10.1016/j.apsoil.2015.10.021.

Adnan, M., Xiao, B., Xiao, P., Zhao, P., Li, R., Bibi, S., 2022. Research progress on heavy metals pollution in the soil of smelting sites in China. Toxics 10, 231. https://doi.org/10.3390/toxics10050231.

Ahmad, M., Ok, Y.S., Rajapaksha, A.U., Lim, J.E., Kim, B.Y., Ahn, J.H., Lee, Y.H., Al-Wabel, M.I., Lee, S.E., Lee, S.S., 2016. Lead and copper immobilization in a shooting range soil using soybean stover- and pine needle-derived biochars: chemical, microbial and spectroscopic assessments, 15 J. Hazard Mater. 301, 179–186. https://doi.org/10.1016/j.jhazmat.2015.08.029.

Alamri, S.A., Siddiqui, M.H., Al-Khaishany, M.Y., Nasir Khan, M., Ali, H.M., Alaraidh, I.A., Alsahli, A.A., Al-Rabiah, H., Mateen, M., 2018. Ascorbic acid improves the tolerance of wheat plants to lead toxicity. J. Plant Interact. 13, 409–419. https://doi.org/10.1080/17429145.2018.1491067.

Ali, A., Guo, D., Zhang, Y., Sun, X., Jiang, S., Guo, Z., Huang, H., Liang, W., Li, R., Zhang, Z., 2017. Using bamboo biochar with compost for the stabilization and phytotoxicity reduction of heavy metals in mine-contaminated soils of China. Sci. Rep. 7, 2690. https://doi.org/10.1038/s41598-017-03045-9.

Altaf, M.A., Hao, Y., Shu, H., Mumtaz, M.A., Cheng, S., Alyemeni, M.N., Ahmad, P., Wang, Z., 2023. Melatonin enhanced the heavy metal-stress tolerance of pepper by mitigating the oxidative damage and reducing the heavy metal accumulation. J. Hazard. Mater., 131468 https://doi.org/10.1016/j.jhazmat.2023.131468.

Al-Wabel, M.I., Usman, A.R., El-Naggar, A.H., Aly, A.A., Ibrahim, H.M., Elmaghraby, S., Al-Omran, A., 2015. Conocarpus biochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. Saudi J. Biol. Sci. 22 (4), 503–511. https://doi.org/10.1016/j.sjbs.2014.12.003.

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Yang, S., Dong, R., Ali, B., Rahimi, M., 2023. Salinity stress improves antioxidant potential by modulating physio-biochemical responses in Moringa oleifera Lam. Sci. Rep. 13, 2895. https://doi.org/10.1038/s41598-023-29954-6.

Bhuyan, M.H.M.B., Hasanuzzaman, M., Mahmud, J.A., Hossain, M.S., Bhuiyan, T.F., Fujita, M., 2019. Unraveling morphophysiological and biochemical responses of triticum aestivum L. to extreme pH: coordinated actions of antioxidant defense and glyoxalase systems. Plants 18 8 (1), 24. https://doi.org/10.3390/plants8010024.

Bian, F., Zhong, Z., Zhang, X., Yang, C., Gai, X., 2020. Bamboo - An untapped plant resource for the phytoremediation of heavy metal contaminated soils. Chemosphere 246, 125750. https://doi.org/10.1016/j.chemosphere.2019.125750.

Bilska, K., Wojciechowska, N., Alipour, S., Kalemba, E.M., 2019. Ascorbic acid-the little-known antioxidant in woody plants. Antioxidants 14 8 (12), 645. https://doi.org/10.3390/antiox8120645.

Calcan, S.I., Pârvulescu, O.C., Ion, V.A., Răducanu, C.E., Bădulescu, L., Madjar, R., Dobre, T., Egri, D., Moț, A., Iliescu, L.M., Jerca, I.O., 2022. Effects of biochar on soil properties and tomato growth. Agronomy 12 (8), 1824. https://doi.org/10.3390/agronomy12081824.

Cenkci, S., Cigerci, I.H., Yildiz, M., Ozay, C., Bozdag, A., Terzi, H., 2010. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environ. Exp. Bot. 67, 467–473. https://doi.org/10.1016/j.envexpbot.2009.10.001.

Chen, Y.X., Huang, X.D., Han, Z.Y., Huang, X., Hu, B., Shi, D.Z., Wu, W.X., 2010. Effects of bamboo charcoal and bamboo vinegar on nitrogen conservation and heavy metals immobility during pig manure composting, 81 Chemosphere 78 (9), 1177. https://doi.org/10.1016/j.chemosphere.

Chen, X., Zhou, Y., Cong, Y., Zhu, P., Xing, J., Cui, J., Xu, W., Shi, Q., Diao, M., Liu, H.Y., 2021. Ascorbic acid-induced photosynthetic adaptability of processing tomatoes to salt stress probed by fast OJIP fluorescence rise, 16 Front Plant Sci. 12, 594400. https://doi.org/10.3389/fpls.2021.594400.

Dusengemungu, L., Mubemba, B., Gwanama, C., 2022. Evaluation of heavy metal contamination in copper mine tailing soils of Kitwe and Mufulira, Zambia, for reclamation prospects, 4 Sci. Rep. 12 (1), 11283. https://doi.org/10.1038/s41598-022-15458-2.

Emamverdian, A., Ding, Y., 2017. Effects of heavy metals' toxicity on plants and enhancement of plant defense mechanisms of Si-mediation "Review. Int. J. Environ. Agric. Res. 3 (4), 41–51.

Emamverdian, A., Ding, Y., Barker, J., Liu, G., Li, Y., Mokhberdoran, F., 2023. Sodium nitroprusside improves bamboo resistance under Mn and Cr toxicity with stimulation of antioxidants activity, relative water content, and metal translocation and accumulation. Int. J. Mol. Sci. 24, 1942. https://doi.org/10.3390/ijms24031942.

Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y., 2018. Growth responses and photosynthetic indices of bamboo plant (Indocalamus latifolius) under heavy metal stress. Sci. World J. 2018 (1), 1219364.

Fellet, G., Marchiol, L., Vedove, G.D., Peressotti, A., 2011. Application of biochar on mine tailings: effects and perspectives for land reclamation. Chemosphere 83, 1262–1267. https://doi.org/10.1016/j.chemosphere.2011.03.053.

Fiaz, K., Danish, S., Younis, U., Malik, S.A., Raza Shah, M.,H., Niaz1, S., 2014. Drought impact on Pb/Cd toxicity remediated by biochar in Brassica campestris. J. Soil Sci. Plant Nutr. 14, 845–854. https://doi.org/10.4067/S0718-95162014005000067.

Georgiadou, E.C., Kowalska, E., Patla, K., Kulbat, K., Smolińska, B., Leszczyńska, J., Fotopoulos, V., 2018. Influence of heavy metals (Ni, Cu, and Zn) on nitro-oxidative stress responses, proteome regulation and allergen Production in Basil (*Ocimum basilicum* L.) Plants. Front. Plant Sci. 9, 862. https://doi.org/10.3389/fpls.2018.00862.

Ghaderian, S.M., Ghotbi Ravandi, A.A., 2012. Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. J. Geochem Explor 123, 25–32. https://doi.org/10.1016/j.gexplo.2012.06.022.

Ghandali, M.V., Safarzadeh, S., Ghasemi-Fasaei, R., Zeinali, S., 2024. Heavy metals immobilization and bioavailability in multi-metal contaminated soil under ryegrass cultivation as affected by ZnO and MnO2 nanoparticle-modified biochar. Sci. Rep. 14 (1), 10684 https://doi.org/10.1038/s41598-024-61270-5. May 9; PMID: 38724636; PMCID: PMC11082237.

Ghorbani, A., Pishkar, L., Roodbari, N., Pehlivan, N., Wu, C., 2021. Nitric oxide could allay arsenic phytotoxicity in tomato (*Solanum lycopersicum* L.) by modulating photosynthetic pigments, phytochelatin metabolism, molecular redox status and arsenic sequestration. Plant Physiol. Biochem. 167, 337–348. https://doi.org/10.1016/j.plaphy.2021.08.019.

Ghorbani, A., Emamverdian, A., Pehlivan, N., Zargar, M., Razavi, S.M., Chen, M., 2024. Nano-enabled agrochemicals: Mitigating heavy metal toxicity and enhancing crop adaptability for sustainable crop production. J. Nanobiotechnol. 22, 91.

Ghorbani, A., Pishkar, L., Roodbari, N., Ali Tavakoli, S., Moein Jahromi, E., Chu, W., 2022. Nitrate reductase is needed for methyl jasmonate-mediated arsenic toxicity tolerance of rice by modulating the antioxidant defense system, glyoxalase system and arsenic sequestration mechanism. J. Plant Growth Regul. 42, 1107. https://doi.org/10.1007/s00344-022-10616-2.

Gill, R.A., Ali, B., Islam, F., Farooq, M.A., Gill, M.B., Mwamba, T.M., Zhou, W., 2015. Physiological and molecular analyses of black and yellow seeded Brassica napus regulated by 5-aminolivulinic acid under chromium stress. Plant Physiol. Biochem 94, 130–143. https://doi.org/10.1016/j.plaphy.2015.06.001.

Gonzaga, M.I.S., Mackowiak, C.L., Comerford, N.B., Moline, E.F.D.V., Shirley, J.P., Guimaraes, D.V., 2017. Pyrolysis methods impact biosolids derived biochar composition, maize growth and nutrition. Soil Tillage Res 165, 59–65. https://doi.org/10.1016/j.still.2016.07.009.

Hasanuzzaman, M., Hossain, M.A., Fujita, M., 2011. Nitric oxide modulates antioxidant defense andmethylglyoxal detoxification system and reduces salinity induced damage in wheat seedling. Plant, Biotecnol. Rep. 5, 353–365. https://doi.org/10.1007/s11816-011-0189-9.

Hasnain, M., Munir, N., Abideen, Z., Zulfiqar, F., Koyro, H.W., El-Naggar, A., Caçador, I., Duarte, B., Rinklebe, J., Yong, J.W.H., 2023. Biochar-plant interaction and detoxification strategies under abiotic stresses for achieving agricultural resilience: A critical review. Ecotoxicol. Environ. Saf. 249, 114408 https://doi.org/10.1016/j.ecoenv.2022.114408. Jan 1; Epub 2022 Dec 12. PMID: 36516621.

Hlihor, R.M., Roșca, M., Hagiu-Zaleschi, L., Simion, I.M., Daraban, G.M., Stoleru, V., 2022. Medicinal plant growth in heavy metals contaminated soils: responses to metal stress and induced risks to human health. Toxics 10, 499. https://doi.org/10.3390/toxics10090499.

Hmid, A., Chami, Z.A., Sillen, W., Vocht, A.D., Vangronsveld, J., 2015. Olive mill waste biochar: a promising soil amendment for metal immobilization in contaminated soils. Environ. Sci. Pollut. Res 22, 1444–1456. https://doi.org/10.1007/s11356-014-3467-6.

Hosseini, S.M., Rezazadeh, M., Salimi, A., Ghorbanli, M., 2018. Distribution of heavy metals and arsenic in soils and indigenous plants near an iron ore mine in northwest Iran. Acta Ecol. Sin. 38 (5), 363–367. https://doi.org/10.1016/j.chnaes.2018.02.004.

Houben, D., Evrard, L., Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere 92, 1450–1457. https://doi.org/10.1016/j.chemosphere.2013.03.055.

Hu, X.-F., Jiang, Y., Shu, Y., Hu, X., Liu, L., Luo, F., 2014. Effects of mining wastewater discharges on heavy metal pollution and soil enzyme activity of the paddy fields. J. Geochem. Explor. 147, 139–150. https://doi.org/10.1016/j.gexplo.2014.08.001.

Ibrahim, E.A., El-Sherbini, M.A.A., Selim, E.-M.M., 2022. Effects of biochar on soil properties, heavy metal availability and uptake, and growth of summer squash grown in metal-contaminated soil. Sci. Hortic. 301, 111097 https://doi.org/10.1016/j.scienta.2022.111097.

Jung, H.-i, Kong, M.-S., Lee, B.-R., Kim, T.-H., Chae, M.-J., Lee, E.-J., Jung, G.-B., Lee, C.-H., Sung, J.-K., Kim, Y.-H., 2019. Exogenous glutathione increases arsenic translocation into shoots and alleviates arsenic-induced oxidative stress by sustaining ascorbate–glutathione homeostasis in rice seedlings. Front Plant Sci. 10, 1089. https://doi.org/10.3389/fpls.2019.01089.

Kalinke, C., Oliveira, P.R., Oliveira, G.A., Mangrich, A.S., Marcolinojunior, L.H., Bergamini, M.F., 2017. Activated biochar: preparation, characterization and electroanalytical application in an alternative strategy of nickel determination. Anal. Chim. Acta 983, 103–111. https://doi.org/10.1016/j.aca.2017.06.025.

Karimi, N., Shayesteh, L.S., Ghasmpour, H., Alavi, M., 2013. Effects of Arsenic on Growth, Photosynthetic Activity, and Accumulationin Two New Hyperaccumulating Populations of *Isatis cappadocica Desv*. J. Plant Growth Regul. 32, 823–830. https://doi.org/10.1007/s00344-013-9350-8.

Kharbech, O., Sakouhi, L., Ben Massoud, M., Mur, L.A.J., Corpas, F.J., Djebali, W., Chaoui, A., 2020. Nitric oxide and hydrogen sulfide protect plasma membrane integrity and mitigate chromium-induced methylglyoxal toxicity in maize seedlings. Plant Physiol. Biochem. 157, 244–255. https://doi.org/10.1016/j.plaphy.2020.10.017.

Kim, H.-S., Kim, K.-R., Kim, H.-J., Yoon, J.-H., Yang, J.E., Ok, Y.S., Owens, G., Kim, K.-H., 2015. Effect of biochar on heavy metal immobilization and uptake by lettuce (*Lactuca sativa* L.) in agricultural soil. Environ. Earth Sci. 74, 1249–1259. https://doi.org/10.1007/s12665-015-4116-1.

Kotroczó, Z., Veres, Z., Fekete, I., Krakomperger, Z., Tóth, J.A., Lajtha, K., Tóthmérész, T., 2014. Soil enzyme activity in response to long-term organic matter manipulation. Soil Biol. Biochem. 70, 237–243. https://doi.org/10.1016/j.soilbio.2013.12.028.

Lehmann, J., Rillig, M.C., Thies, J., Massielo, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota—A review. Soil Biol. Biochem. 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.

Li, M., Barbaro, E., Bellini, E., Saba, A., Sanità di Toppi, L., Varotto, C., 2020. Ancestral function of the phytochelatin synthase C-terminal domain in inhibition of heavy metal-mediated enzyme overactivation. J. Exp. Bot. 71, 6655–6669. https://doi.org/10.1093/jxb/eraa386.

Li, Y., Shen, F., Guo, H., Wang, Z., Yang, G., Wang, L., Zhang, Y., Zeng, Y., Deng, S., 2015. Phytotoxicity assessment on corn stover biochar, derived from fast pyrolysis, based on seed germination, early growth, and potential plant cell damage. Environ. Sci. Pollut. Res Int 22 (12), 9534–9543. https://doi.org/10.1007/s11356-015-4115-5.

Liu, N., Lin, Z., Guan, L., Gaughan, G., Lin, G., 2014. Antioxidant enzymes regulate reactive oxygen species during pod elongation inPisum sativum and Brassica chinensis. PLoS ONE 9, e87588. https://doi.org/10.1371/journal.pone.0087588.

Lu, H., Li, Z., Fu, S., Méndez, A., Gascó, G., Paz-Ferreiro, J., 2014. Can biochar and phytoextractors be jointly used for cadmium remediation?, 16 PLoS One 9 (4), e95218. https://doi.org/10.1371/journal.pone.0095218.

Lu, K., Yang, X., Gielen, G., Bolan, N., Ok, Y.S., Niazi, N.K., Xu, S., Yuan, G., Chen, X., Zhang, X., Liu, D., Song, Z., Liu, X., Wang, H., 2017. Effect of bamboo and rice straw biochars on the mobility and redistribution of heavy metals (Cd, Cu, Pb and Zn) in contaminated soil. J. Environ. Manag. 186, 285–292. https://doi.org/10.1016/j.jenvman.2016.05.068.

Lucchini, P., Quilliam, R.S., Deluca, T.H., Vamerali, T., Jones, D.L., 2014. Increased bioavailability of metals in two contrasting agricultural soils treated with waste wood-derived biochar and ash. Environ. Sci. Pollut. Res Int21 3230–3240. https://doi.org/10.1007/s11356-013-2272-y.

Ma, J.W., Wang, F.Y., Huang, Z.H., Wang, H., 2010. Si-multaneous removal of 2,4-dichlorophenol and Cdfrom soils by electrokinetic remediation combinedwith activated bamboo charcoal. J. Hazard. Mater. 176, 715–720.

Mackie, K.A., Marhan, S., Ditterich, F., Schmidt, H.P., Kandeler, E., 2015. The effects of biochar and compost amendments on copper immobilization and soil microorganisms in a temperate vineyard. Agric. Ecosyst. Environ. 201, 58–69. https://doi.org/10.1016/j.agee.2014.12.001.

Mahar, A., Wang, P., Ali, A., Guo, Z., Awasthi, M.K., Lahori, A.H., Wang, Q., Shen, F., Li, R., Zhang, Z., 2016. Impact of CaO, fly ash, sulfur and Na_2S on the (im) mobilization and phytoavailability of Cd, Cu and Pb in contaminated soil. Ecotoxicol. Environ. Saf. 134, 116–123. https://doi.org/10.1016/j.ecoenv.2016.08.025.

Marchand, L., Pelosi, C., González-Centeno, M.R., Maillard, A., Ourry, A., Galland, W., Teissedre, P.L., Bessoule, J.J., Mongrand, S., Morvan-Bertrand, A., Zhang, Q., Grosbellet, C., Bert, V., Oustrière, N., Mench, M., Brunel-Muguet, S., 2016. Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol. Chemosphere 156, 150–162. https://doi.org/10.1016/j.chemosphere.2016.04.129.

Mohi-Ud-Din, M., Siddiqui, N., Rohman, M., Jagadish, S.V.K., Ahmed, J.U., Hassan, M.M., Hossain, A., Islam, T., 2021. Physiological and biochemical dissection reveals a trade-off between antioxidant capacity and heat tolerance in bread wheat (*Triticum aestivum* L.). Antioxidants 10, 351. https://doi.org/10.3390/antiox10030351.

Mourato, M., Moreira, I., Leit ão, I., Pinto, F., Sales, J., Martins, L., 2015. Effect of Heavy Metals in Plants of the *Genus Brassica*. Int. J. Mol. Sci. 16 (8), 17975–17998. https://doi.org/10.3390/ijms160817975.

Mousavi, S.R., Niknejad, Y., Fallah, H., Tari, D.B., 2020. Methyl jasmonate alleviates arsenic toxicity in rice. Plant Cell Rep. 39, 1041–1060. https://doi.org/10.1007/s00299-020-02547-7.

Ogawa, S., Yoshidomi, T., Yoshimura, E., 2011. Cadmium (II)-stimulated enzyme activation of Arabidopsis thaliana phytochelatin synthase 1. J. Inorg. Biochem. 105, 111–117. https://doi.org/10.1016/j.jinorgbio.2010.09.011.

Olmo, M., Alburquerque, J.A., Barrón, V., Del Campillo, M.C., Gallardo, A., Fuentes, M., Villar, R., 2014. Wheat growth and yield responses to biochar addition under mediterranean climate conditions. Biol. Fert. Soils 50, 1177–1187. https://doi.org/10.1007/s00374-014-0959-y.

Ouyang, X.K., Jin, R.N., Yang, L.P., Wang, Y.G., Yang, L.Y., 2014. Bamboo derived porous bio adsorbents and their adsorption of Cd (II) from mixed aqueous solutions. RSC Adv. 4 (54), 28699–28706. https://doi.org/10.1039/C4RA03422H.

Pathan, S.I., Žifčáková, L., Ceccherini, M.T., Pantani, O.L., Větrovský, T., Baldrian, P., 2017. Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in coniferous forest soil. Soil Biol. Biochem. 105, 71–80. https://doi.org/10.1016/j.soilbio.2016.11.003.

Pehlivan, N., Gedik, K., Wang, J.J., 2023. Tea-based biochar-mediated changes in cation diffusion homeostasis in rice grown in heavy metal (loid) contaminated mining soil. Plant Physiol. Biochem., 107889

Pehlivan, N., Gedik, K., Eltem, R., Terzi, E., 2021. Dynamic interactions of *Trichoderma harzianum* TS 143 from an old mining site in Turkey for potent metal (oid) s phytoextraction and bioenergy crop farming. J. Hazard. Mater. 403, 123609 https://doi.org/10.1016/j.jhazmat.2020.123609.

Pehlivan, N., Wang, J.J., 2022. Transcriptional insights into Cu related tolerance strategies in maize linked to a novel tea-biochar. Environ. Pollut. 293, 118500 https://doi.org/10.1016/j.envpol.2021.118500.

Rai, G.K., Bhat, B.A., Mushtaq, M., Tariq, L., Rai, P.K., Basu, U., Dar, A.A., Islam, S.T., Dar, T.U.H., Bhat, J.A., 2021. Insights into decontamination of soils by phytoremediation: a detailed account on heavy metal toxicity and mitigation strategies. Physiol. Plant. 173, 287–304. https://doi.org/10.1111/ppl.13433.

Raihan, M.R.H., Rahman, M., Mahmud, N.U., Adak, M.K., Islam, T., Fujita, M., Hasanuzzaman, M., 2022. Application of Rhizobacteria, Paraburkholderia fungorum and Delftia sp. Confer Cadmium Tolerance in Rapeseed (*Brassica campestris*) through modulating antioxidant defense and glyoxalase Systems. Plants 11, 2738. https://doi.org/10.3390/plants11202738.

Rezayian, M., Ebrahimzadeh, H., Niknam, V., 2023. Metabolic and physiological changes induced by nitric oxide and its impact on drought tolerance in soybean. J. Plant Growth Regul. 42, 1905–1918. https://doi.org/10.1007/s00344-022-10668-4.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidant defence mechanism in plants under stressful conditions. J. Bot. 2012, 217037 https://doi.org/10.1155/2012/217037.

Shen, X., Huang, D.Y., Ren, X.F., Zhu, H.H., Wang, S., Xu, C., He, Y.B., Luo, Z.C., Zhu, Q.H., 2016. Phytoavailability of Cd and Pb in crop straw biochar-amended soil is related to the heavy metal content of both biochar and soil. J. Environ. Manag. 168, 245–251. https://doi.org/10.1016/j.jenvman.2015.12.019.

Shetty, R., Prakash, N.B., 2020. Effect of different biochars on acid soil and growth parameters of rice plants under aluminium toxicity. Sci. Rep. 10, 12249 https://doi.org/10.1038/s41598-020-69262-x.

Souri, Z., Karimi, N., Norouzi, L., Ma, X., 2020. Elucidating the physiological mechanisms underlying enhanced arsenic hyperaccumulation by glutathione modified super paramagnetic iron oxide nanoparticles in *Isatis cappadocica*. Ecotoxicol. Environ. Saf. 206, 111336 https://doi.org/10.1016/j.ecoenv.2020.111336.

Sytar, O., Kumar, A., Latowski, D., Kuczynska, P., Strzalka, K., Prasad, M.N.V., 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol. Plant 35, 985–999. https://doi.org/10.1007/s11738-012-1169-6.

Upadhyay, G., Tiwari, N., Maurya, H., Upadhyay, J., Joshi, R., Ansari, M.N., 2021. In vivo wound-healing and antioxidant activity of aqueous extract of Roylea elegans leaves against physically induced burn model in Wistar albino rats. 3 Biotech 11, 442. https://doi.org/10.1007/s13205-021-02993-4.

Vezza, M.E., Luna, D.F., Agostini, E., Talano, M.A., 2019. Glutathione, a key compound for As accumulation and tolerance in soybean plants treated with AsV and AsIII. Environ. Exp. Bot. 162, 272–282. https://doi.org/10.1016/j.envexpbot.2019.03.002.

Wang, Y., Pan, F., Wang, G., Zhang, G., Wang, Y., Chen, X., Mao, Z., 2014. Effects of biochar on photosynthesis and antioxidative system of Malus hupehensis Rehd. seedlings under replant conditions. Sci. Hortic. 175, 9–15. https://doi.org/10.1016/j.scienta.2014.05.029.

Wang, Q., Wang, Z., Awasthi, M.K., Jiang, Y., Li, R., Ren, X., Zhao, J., Shen, F., Wang, M., Zhang, Z., 2016. Evaluation of medical stone amendment for the reduction of nitrogen loss and bioavailability of heavy metals during pig manure composting. Bioresour. Technol. 220, 297–304. https://doi.org/10.1016/j.biortech.2016.08.081.

Wu, D., Saleem, M., He, T., He, G., 2021. The mechanism of metal homeostasis in plants: a new view on the synergistic regulation pathway of membrane proteins, lipids and metal ions, 15 Membr. (Basel) 11 (12), 984. https://doi.org/10.3390/membranes11120984.

Xian, Y., Wang, M., Chen, W., 2015. Quantitative assessment on soil enzyme activities of heavy metal contaminated soils with various soil properties. Chemosphere 139, 604–608. https://doi.org/10.1016/j.chemosphere.2014.12.060.

Xu, Z.Y., Tang, M., Chen, H., Ban, Y.H., Zhang, H.H., 2012. Microbial community structure in the rhizosphere of Sophora viciifolia grown at a lead and zinc mine of northwest China, 453–464 Sci. Total Environ. 435–436. https://doi.org/10.1016/j.scitotenv.2012.07.029.

Yang, X., Liu, J., McGrouther, K., Huang, H., Lu, K., Guo, X., He, L., Lin, X., Che, L., Ye, Z., Wang, H., 2016. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. Environ. Sci. Pollut. Res Int 23 (2), 974–984. https://doi.org/10.1007/s11356-015-4233-0.

Yusefi-Tanha, E., Fallah, S., Pokhrel, L.R., Rostamnejadi, A., 2023. Addressing global food insecurity: soil-applied zinc oxide nanoparticles promote yield attributes and seed nutrient quality in Glycine max L, 11 Sci. Total Environ. 876, 162762. https://doi.org/10.1016/j.scitotenv.2023.162762.

Zha, Y., Zhao, B., Niu, T., 2022. Bamboo Biochar and Zinc Oxide Nanoparticles Improved the Growth of Maize (*Zea mays* L.) and Decreased Cadmium Uptake in Cd-Contaminated Soil. Agriculture 12, 1507. https://doi.org/10.3390/agriculture12091507.

Zhang, M., Riaz, M., Zhang, L., El-Desouki, Z., Jiang, C., 2019. Biochar induces changes to basic soil properties and bacterial communities of different soils to varying degrees at 25 mm rainfall: more effective on acidic soils, 12 Front Microbiol 10, 1321. https://doi.org/10.3389/fmicb.2019.01321.

Zulfiqar, U., Haider, F.U., Ahmad, M., Hussain, S., Maqsood, M.F., Ishfaq, M., Shahzad, B., Waqas, M.M., Ali, B., Tayyab, M.N., Ahmad, S.A., Khan, I., Eldin, S.M., 2023. Chromium toxicity, speciation, and remediation strategies in soil-plant interface: a critical review. Front. Plant Sci. 13, 1081624 https://doi.org/10.3389/fpls.2022.1081624.