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Research Article

Effect of zeolite, biochar, and mycorrhiza on phytoremediation potential of forage Amaranth (*Amaranthus caudatus* L.) in lead contaminated soil

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Abstract

High absorption and accumulation of lead in the cultivated *Amaranthus caudatus* L. make this plant suitable for the purpose of phytoremediation of lead-contaminated soils. The present factorial experiment was carried out based on a completely randomized design with three replications in the Greenhouse of the Faculty of Agriculture, Lorestan University, during 2019. The factors considered in the study included zeolite (0, 5 and 10%), biochar (0, 7.5 and 15%), and mycorrhizal fungi (no mycorrhiza and use of mycorrhiza). The highest plant height (1.93 m) and root dry weight (2.55 g) were obtained in the combined treatment of not using zeolite, biochar 15% by weight, and mycorrhiza. Also, the highest biological yield (54.05 g) and accumulated lead in roots (20.05 mg/kg) were obtained in the combined treatment of zeolite 5%, biochar 15%, and no mycorrhiza. Moreover, the highest number of sub-branches (26.67) was recorded in the combined treatment of zeolite 10% + no biochar + no mycorrhiza ($Z_2B_0M_0$). Furthermore, the highest concentrations of lead in the soil around roots (49.92 mg/kg) were observed in the treatment consisting of zeolite 5% + biochar 7.5% + mycorrhiza. Findings also showed the highest activities of catalase, peroxidase, and superoxide dismutase enzymes in the combined treatment of the plants with zeolite 10% + biochar 15% + no mycorrhiza were obtained .On the other hand, the highest activities of ascorbate peroxidase and glutathione reductase were recorded in the combined treatments of zeolite 5% + biochar 7.5% + no use of mycorrhiza and zeolite 10% + biochar 15% + the use of mycorrhiza, respectively. The highest concentration of lead in leaves (17.71 mg/kg) was obtained in none zeolite + none mycorrhiza + biochar 15%, which was among the best treatments of the study resulting in a high biological performance of the plants (41.55 g). Finally, the most favorable treatment with the highest biological yield (54.05 g) and maximum lead contents of the roots (20.05 mg/kg) was obtained by zeolite 5% + biochar 15% + none mycorrhiza. The maximum biological yield of the plant (54.05 g) and the highest concentration of lead in roots (20.05 mg/kg) were observed in the combined treatment of zeolite 5%, biochar 15%, and no mycorrhiza ($Z_1B_2M_0$). This is the best treatment for phytoremediation, in which the plants absorb the highest concentration of lead. On the other hand, the highest accumulation of lead in leaves (17.71 mg/kg) was obtained in the treatment with no zeolite and mycorrhiza + using biochar 15% ($Z_0B_2M_0$). In view of the high biological performance of *Amaranthus caudatus* L. in this treatment (41.55 g), it is considered one of the most effective plants in bioaccumulation.

Keywords: Antioxidant enzymes, Biological yield, Heavy metals, Phytoremediation, Soil contamination

Introduction

Releasing industrial and urban sewage into water resources and agricultural lands, as well as the application of pesticides and chemical fertilizers containing heavy metals pollute agricultural lands with heavy metals. Accumulation of these metals in plant and animal organs causes serious damage to their health and that of the organisms such as humans that feed on them (Safari Aman *et al*., 2016). Among these heavy metals, lead, cadmium, and arsenic are not necessary for metabolism, and they are harmful to the body even at small levels. Lead causes conditions such as disturbances in hemoglobin biosynthesis and anemia, kidney damage, increased blood pressure, miscarriage, male infertility, nervous system disorders, brain damage, etc. (Karbasi *et al*., 2010). Owing to their chemical stability, poor degradability, and high bioaccumulation potential, heavy elements can damage and pose ecological risks to organisms at different levels of the food chain (Ling *et al*., 2008; European Commission, 2013). On the other hand, considering the water crisis in recent years and the need to preserve the existing water resources and control water shortage in the country, it is necessary to filter sewage as a source of irrigation water to manage water shortage. Redemption of contaminated soils is difficult and

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traditional methods are often costly and impossible to use on a large scale (Safari Aman *et al*., 2016).

Phytoremediation is potentially a viable and cost-effective solution for soils contaminated with heavy metal. Certain plants can remove high amounts of heavy metals from the soil (Karbassi and Moattar, 2008). A range of bioremediation technologies including phytodegredation, phytostabilization, phytoextraction, phytovolatilization, rhizosofiltration etc. are available to clean the environment from pollutants such as heavy metals (yang *et al*., 2005; Gajic and Pavlovic, 2018). Deciding on the right plant for phytoremediation of the soils depends on the climatic conditions, the level and type of contaminant, the range of tolerance of the plant to the contaminant, and the transfer of the metal from plant roots to shoots (Rostami, 2017).

Another recent approach to mitigate the adverse effects of heavy metals in soils is the use of mineral absorbents. Sepiolite and zeolite are low-cost minerals with high capacity to absorb heavy metals (Ansari Mahabadi, 2003). Clay minerals control the contaminants through absorption and surface absorption. Zeolites and bentonites have been identified as effective materials to absorb heavy metals owing to their specific surface area and high cation exchange capacity, low cost, and availability (Esmaeilpour Fard *et al*., 2015). Applying zeolite to modify the contaminated soil was reported to significantly reduce the available form of zinc in soils (Castaldi *et al*., 2005).

Among the most effective methods to mitigate the adverse effects of heavy metals and stabilize contaminated soils is the use of organic and inorganic modifying substances. Adding biochar as an amendment to the soil may modify its chemical properties and provide suitable conditions for the immobilization of heavy metals (Boostani and Najafighiri, 2018). Surface functional groups and surface adsorption sites in biochar can increase the cation exchange capacity of soil (Paz-Ferreiro *et al*., 2014). In their studies on corn plants, Haji Najafi *et al*. (2016) showed that biochar had significant effects on the dry weight of shoots and roots and the concentration of lead in stems, roots, and leaves.

The efficiency of phytoremediation is also improved through symbiotic relationships between beneficial soil microorganisms and plants. Mycorrhizal fungi can play an important role in phytoremediation of heavy metals (Leyval *et al*., 1997). An increase in hyphae production and sporulation of the fungus *Glomus intraradices* has been observed at high concentrations of heavy metals. Vesicular-arbuscular mycorrhiza or intracellular mycorrhiza (VAM fungi) reduce the toxicity of heavy metals (Leyval *et al*., 1997). Improving the symbiotic relationship between mycorrhizal fungi and plant roots leads to an increase in the absorption of heavy metals; Therefore, any factor that improves and increases this symbiotic relationship will certainly increase the efficiency of phytoremediation (Zare *et al*., 2006).

Amaranthus caudatus L. is a broad-leaved plant that is considered as a new agricultural plant in many regions of the world, including Iran. Being resistant to unfavorable conditions such as soils poor in nutrients and a wide range of abiotic stresses such as heat, radiation, and drought has made it possible to use this plant as a nutritious green product in semi-arid regions (Ansari Ardali and Agha Alikhani, 2015). The plant produces a large amount of fodder in a short period of time, and because of its high biomass and lead absorption rate, it is very suitable for remediation of soils contaminated with this heavy metal. The average absorption of lead in its roots is higher than in its aerial organs (Akbarpour Saraskanroud *et al*., 2012). Plants such as *Lolium*, wild *Amaranthus*, and *Sorghum* effectively reduced the concentration of heavy metals in polluted soils in the Urmia region (Nejatzadeh and Gholami-Borujeni, 2017). Therefore, considering the increasing levels of heavy metals in soil and the use of urban and industrial wastewater, this research was an attempt to investigate the distribution and accumulation of lead in *Amaranthus caudatus* L. plants and the soil by applying different treatments.

Materials and methods

A factorial experiment in the form of a complete randomized design with three replications was conducted in plastic pots (50 cm height and 25 cm diameter) containing 15 kg of dry clay loam soil in the Greenhouse of the Faculty of Agriculture, Lorestan University (45°, 17’ East and 33°, 26’ North, 1210 meters above sea level). The treatments included zeolite in three levels (control, 5 and 10% by weight of the pot soil), wheat straw biochar in three levels (control, 7.5 and 15% by weight of the pot soil), and two levels of mycorrhiza fungus (inoculation and non-inoculation). Two pots were considered for each treatment combination. The soil was analyzed before applying the treatments (Table 1), following air drying and passing through a 2 mm sieve to determine lead, nitrogen, phosphorus, and potassium contents, as well as lime percentage, soil texture (using the hydrometric method), pH, and electrical conductivity (EC).

Treatments abbreviations are as follows: Z_0: no use of zeolite; Z_1: use of zeolite 5% by weight of the pot; Z_2: use of zeolite 10% by weight of the pot; B_0: no use of biochar; B_1: use of biochar 7.5% by weight of the pot; B_2: use of biochar 15% by weight of the pot; M0: no use of mycorrhiza; and M_1: use of mycorrhiza.

Before sowing, 15 kg of soil was weighed and poured into each plastic bag. In order to introduce heavy metal pollution, 200 mg/kg of lead was added in the form of $Pb(NO_3)_2$ dissolved in distilled water by spraying the solution evenly and layer by layer on the surface of the soil inside bags. The contents of the bags were left intact for one month to stabilize the lead in the soil. Also, before sowing the seeds, zeolite was added at three levels: zero (control), 5 and 10% by weight of the pot soil. Moreover, three levels of biochar (0, 7.5 and 15% by weight of the pot soil) were mixed with the soil. In the next step, the soils supplemented with lead,

Table 1. Physicochemical analysis of the soil sampled from the study area

Sampling depth (cm)	Soil texture	Clay (%)	Silt (%)	Sand (%)	Absorbable potassium (mg kg^{-1})	Absorbable phosphorus (mg kg^{-1})	Nitrogen (%)	Organic carbon (%)	pH	Electrical conductivity (dS m^{-1})
0-30	loam	7.2	44.7	48.1	382	7.9	0.028	0.234	8.7	0.419

zeolite, and/or biochar were inoculated with mycorrhizal fungi (*Glomus intraradices*). To apply mycorrhizal treatments, 5-7 cm of the upper part of the soil in each pot was removed, and a thin, uniform layer of the inoculum (50 g) was spread on the substrate before it was covered with soil again. Finally, 5 seeds of *Amaranthus caudatus* L. were sown in each bag in May 2020. At the end of the growth period, samples were prepared from the leaves, roots, and soil around the roots (rhizosphere) of each pot and taken to the laboratory to assay the heavy metal contents.

Traits assay: At the end of the growth (harvest) period, a number of traits were assayed (103 day). The plant height (m) was measured based on the mean height of 2 plants from each replicate using a meter. In addition, the number of sub-branches was estimated based on the average number of sub-branches of two plants from each replicate, and biological yield (total dry matter performance of each plant) were assayed in the study. In order to measure the dry weight of each plant and its components at the time of harvest, 2 plants were randomly selected, and leaves, flowers, stems, and roots were separated. Then, to determine the dry weight, samples were placed in paper bags in an electric oven set at 80 °C for 48 hours. To determine the dry weight of the roots per plant (g), the plants were removed from the pot and the roots were completely washed to remove soil particles and then transported to the laboratory. The samples were placed in paper envelopes, placed in an electric oven (80 °C) for 48 hours, and then weighed. The mean dry weight of the roots of 2 plants was considered as the dry weight of the roots per plant. In order to determine the amount of lead at the end of the plant growth period, leaf, root, and the soil around roots (rhizosphere) of each pot were sampled which were then taken to the laboratory to measure their lead content. The concentration of lead in the soil was measured using soil digestion through oxidation method (Ryan *et al*., 2001). To measure the absorbable concentration of lead in soil, DTPA extractor was used along with calcium chloride and triethanolamine solution (Lindsay and Norvell, 1978). The concentration of lead in roots and shoots (leaves) was determined using the method of burning and digestion in hydrochloric acid. In addition, dry ash extraction method was used to measure lead. For this purpose, 2 grams of each plant sample were transferred into ceramic crucibles, which were placed in an electric furnace set at 550 °C for 2 hours. Afterwards, 5 ml of 2 N hydrochloric acid was added to the samples and the solutions were slightly heated on an electric stove. Then boiling distilled water was added before the solution was filtered into a 50 ml conical flask using Whatman No. 42 filter paper (Pirzad *et al*., 2008). Finally, the concentration of lead in the extracts was read by the atomic absorption spectrophotometer (240AA, Agilent Co., USA).

Enzymes assay: Leaf samples were used to assay enzyme activities at full flowering stage. The activity of superoxide dismutase (SOD) was measured through spectrophotometric method and based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) at a wavelength of 560 nm (Beauchamp and Fridovich, 1971). In order to extract the enzyme, 0.1 g of leaf tissue, which was previously prepared and stored in the freezer, was poured into 1 ml of the extraction buffer and mixed. The mixture was passed through a thin linen cloth and the resulting extract was centrifuged at 14000 rpm under 4 °C for 20 minutes. The transparent supernatant (enzyme extract) was then separated carefully.

Catalase (CAT) enzyme activity was assayed using a spectrophotometer at 25 °C. The device was set to a wavelength of 240 nm. The solution included 3000 µl of phosphate buffer 50 mM (pH=7), 100 µl of enzyme extract, and 5 µl of hydrogen peroxide (H_2O_2) 30%. The enzyme activity was read for 2 minutes in 20 second intervals (Aebi, 1984).

Peroxidase (POD) activity was measured using the method of Dhindsa *et al*. (1981). Fifty (50) µl of the extracts were mixed with 1 ml of catalase measurement solution containing 50 mM of potassium phosphate buffer (pH=7) and 15 mM of hydrogen peroxide. Then, its absorbance was read at a wavelength of 240 nm for one minute with a spectrophotometer. One enzyme unit of catalase was considered as equal to the decomposition of 1 ml of H_2O_2 per minute.

Ascorbate peroxidase (APX) activity was measured following the method of Ranieri *et al*. (2001). As a result of the reaction between APX, ascorbic acid, and H_2O_2, dehydroascorbate is produced, which is read at a wavelength of 290 nm. The reaction medium contained 600 µl of 0.1 mM EDTA, 1500 µl of 50 mM phosphate buffer (pH=7), 400 µl of 0.5 mM ascorbic acid, 400 µl of 30% H_2O_2, and 100 µl of enzyme extract. Enzyme activity assay was recorded during 4 minutes. With the passage of reaction time, an increase in the amount of absorption was observed.

Glutathione reductase (GR) activity was measured with spectrophotometer following Foyer and Halliwell (1976). Leaf tissue (0.1 g) which was previously prepared and stored under 80 °C condition, was mixed with 1 ml of the extraction buffer. The mixture was then filtered, and the resulting extract was centrifuged for 20 minutes at 4 °C and by 14,000 rpm. Then, the supernatant (enzyme extract) was carefully separated.

Data analysis: MSTAT-C software was used to analyze the data and LSD test was used to compare the means. Correlations between the traits under

investigation were determined based on Pearson's correlation coefficient using SPSS software. The correlation values were evaluated as follows (Rezaei, 2016): $r \leq 0.2$: very weak and insignificant, $0.2 < r \leq 0.4$: weak correlation, $0.4 < r \leq 0.6$: average correlation, $0.6 < r \leq 0.8$: strong correlation, and $r > 0.8$: very strong correlation.

Results and discussion

Plant height: The highest plant height (1.93 m) was obtained in without zeolite + biochar 15% + using mycorrhiza ($Z_0B_2M_1$) in lead-contaminated soil while the lowest plant height (0.44 m) corresponded to the treatment with zeolite 10% + biochar 7.5% + mycorrhiza consumption ($Z_2B_1M_1$) in lead-contaminated soil (Table 2). The $Z_0B_2M_1$ resulted in a 32.19% increase in the height of *Amaranthus* plants compared to the control (Table 3). The application of biochar (15%) seemed to have the greatest effect on increasing the height of the plant, which might be attributed to the increase in the absorption of nutrients, since the absorption of nutrients by affecting the process of photosynthesis and cell division leads to an increase in vegetative growth and the green surface of the plant (Barker and Pilbeam, 2015). Also, mycorrhizal fungi, in addition to helping to improve the mineral nutrition of plants, also increases the growth of plants in soybean and corn through physiological processes (Kapoor *et al*., 2002). Arbuscular mycorrhizal fungi (AMF) can form a mutualistic relationship with 80% of terrestrial plants, in which the extensive extraradical mycelium in the soil enhances immobile nutrient (such as P and Zn) uptake (Smith *et al*., 2003). Also, higher gas exchange has been found in mycorrhiza fungi (Brown *et al*., 1987). In their study on soybean and corn plants, Haidarpour Sarami (2022) showed that the height of both plants was affected by mycorrhiza.

There was a strong, significant positive correlation between the plant height and number of leaves and its biological performance ($0.6 \leq r \leq 0.8$). Also, a moderate significant positive correlation ($0.4 \leq r \leq 0.6$) was found between the plant height and its root dry weight. Moreover, as shown in Table 4, the correlation between the plant height and the number of sub-branches and lead content of the root rhizosphere was positive and weakly significant ($0.2 \leq r \leq 0.4$). This shows that the less lead has entered to plant and remained in soil, the higher its growth and the number of branches.

Number of sub-branches: The highest number of sub-branches (26.67) was recorded under zeolite 10% and non-application of biochar and mycorrhiza ($Z_2B_0M_0$) in lead-contaminated soil, while the lowest number (3) was obtained in the treatment with zeolite 5% non-application of biochar and applying mycorrhiza ($Z_1N_0M_1$) in lead-contaminated soil (Table 2). Zeolite alone in ($Z_2B_0M_0$) treatment increased the number of sub-branches of *Amaranthus caudatus* L. by 48.11% compared to the control (Table 3). Zeolite 10% by weight appears to have had the highest impact on the number of sub-branches. Consumption of biochar and mycorrhiza did not have a considerable effect. Ghasem Khanloo *et al*. (2009) reported that plant branches as a vegetative trait are severely affected by the genetic characteristics of the cultivars, and that, except for plant density; This trait may also be less influenced by the other environmental factors. A positive and moderately significant correlation ($0.4 < r \leq 0.6$) was observed between the number of sub-branches and root dry weight. Also, a positive and weakly significant correlation ($0.2 < r \leq 0.4$) was found between the number of sub-branches and plant height and its biological performance (Table 4).

Plant biological yield: Comparison of the means showed that the highest biological yield of the plant (54.05 g) was related to the treatment including zeolite 5%, biochar 15%, and none consumption of mycorrhiza ($Z_1B_0M_0$) in lead-contaminated soil while the lowest level (2.69 g) belonged to the treatment with zeolite 10%, without biochar and application of mycorrhiza ($Z_2B_0M_1$) in lead-contaminated soil (Table 2). The interaction effects of mycorrhiza, biochar, and maximum zeolite in ($Z_1B_2M_0$) treatment increased the biological performance of the plants by 194.23% compared to the control (Table 3). Biochar (15%) and zeolite (5%) appear to have increased the biological yield of the plant. The combined consumption of biochar and zeolite has increased biological yield by improving the activity of soil microorganisms and the production of plant growth regulators, as well as providing more nutrients. Tahami *et al*. (2014) believe that providing nutrients and improving growth conditions increases the growth in all plant organs. The results of the study by Haji Najafi *et al*. (2016) showed a significant effect of biochar on dry weights of shoots and roots and lead concentration in stems, roots, and leaves. Moreover, zeolite increases the uptake of nutrients through the expansion of the root system and availability of nutrients, ultimately increasing the plant (Farhadi *et al*., 2017). Biological fertilizers improve plant growth and yield through nitrogen stabilization and contribution to releasing nutrients in the soil, production of growth promoting hormones, and increasing root absorption efficiency (Basu *et al*., 2008). Tofighi *et al*. (2016) reported that mycorrhiza fungi can increase the height and general growth in plants and increase the level of nutrients in the plant through changes in hormone levels and the secretion of growth promoting factors and increasing nutrient uptakes. A strong, significant, and positive correlation ($0.6 < r \leq 0.8$) was found between the biological yield, plant height and dry weight of roots in this study; The correlation between biological performance of the plant and lead concentration of roots was positive and moderately significant ($0.4 < r \leq 0.6$), and a poor, significant, and positive correlation ($0.2 < r \leq 0.4$) was found between the biological yield of the plant and the number of sub-branches, lead concentration of the rhizosphere soil, and the lead content of leaves (Table 4).

Table 2. The effects of zeolite, biochar, and mycorrhiza on some traits of *Amaranthus caudatus* L. in lead-contaminated soil

Treatment combination	Plant height (m)	Number of sub-branches per plant	Biological performance in plant (g)	Root dry weight per plant (g)	Lead in rhizosphere soil (mg/kg)	Lead in the root (mg/kg)
$Z_0B_0M_0$	1.46^{b-e*}	18.00^{b}	18.37^{e-g}	2.09^{a-c}	22.16^{d}	12.03^{b-e}
$Z_0B_0M_1$	1.35^{b-g}	10.00^{de}	15.80^{gh}	1.45^{a-f}	37.40^{a-d}	13.89^{ab-e}
$Z_0B_1M_0$	1.50^{a-e}	14.33^{b-d}	4.17^{ij}	0.51^{ef}	42.55^{a-c}	8.03^{e}
$Z_0B_1M_1$	1.36^{b-f}	10.83^{de}	24.42^{d-f}	1.33^{a-f}	41.17^{a-c}	17.81^{ab}
$Z_0B_2M_0$	1.29^{d-g}	16.50^{bc}	41.55^{b}	1.67^{a-e}	39.69^{a-c}	16.36^{a-d}
$Z_0B_2M_1$	1.93^{a}	18.00^{b}	46.37^{ab}	2.55^{a}	41.65^{a-c}	14.36^{a-d}
$Z_1B_0M_0$	0.92^{g-i}	10.00^{de}	8.18^{h-j}	0.69^{d-f}	42.58^{a-c}	15.71^{a-d}
$Z_1B_0M_1$	0.93^{fg-i}	3.00^{g}	6.26^{ij}	0.68^{d-f}	27.80^{cd}	13.42^{a-d}
$Z_1B_1M_0$	1.14^{e-h}	10.67^{de}	27.73^{cd}	1.53^{a-f}	32.63^{a-d}	10.46^{de}
$Z_1B_1M_1$	1.31^{c-g}	8.00^{e-g}	8.34^{h-j}	0.66^{d-f}	49.92^{a}	14.50^{a-d}
$Z_1B_2M_0$	1.75^{ab}	10.67^{de}	54.05^{a}	2.37^{ab}	46.20^{ab}	20.05^{a}
$Z_1B_2M_1$	1.70^{a-d}	11.50^{c-e}	33.07^{c}	0.95^{c-f}	39.66^{a-c}	12.66^{b-e}
$Z_2B_0M_0$	1.36^{b-f}	26.67^{a}	26.33^{c-e}	1.86^{a-d}	37.26^{a-d}	16.94^{a-c}
$Z_2B_0M_1$	0.61^{ij}	3.67^{fg}	2.69^{j}	0.29^{f}	27.59^{cd}	11.01^{c-e}
$Z_2B_1M_0$	1.75^{a-c}	8.33^{ef}	31.00^{cd}	2.27^{ab}	33.27^{a-d}	11.99^{b-e}
$Z_2B_1M_1$	0.44^{j}	8.67^{ef}	2.77^{j}	0.52^{ef}	28.23^{cd}	11.30^{c-e}
$Z_2B_2M_0$	1.07^{e-h}	9.67^{de}	11.67^{g-i}	1.25^{b-f}	30.13^{b-d}	10.42^{de}
$Z_2B_2M_1$	0.77^{h-j}	17.50^{b}	17.83^{fg}	1.69^{a-e}	30.89^{b-d}	16.47^{a-d}

Continued of table 2.

Treatment combination	Lead in leaf (mg/kg)	Catalase	Peroxidase	superoxide dismutase	Ascorbate peroxidase	Glutathione reductase
		(U mg^{-1} protein)				
$Z_0B_0M_0$	6.78^{b-d*}	1.23^{de}	0.54^{d-g}	5.25^{cd}	0.42^{cd}	241.9^{d}
$Z_0B_0M_1$	10.37^{bc}	1.09^{de}	0.50^{e-h}	3.34^{ef}	0.36^{de}	195.3^{ef}
$Z_0B_1M_0$	9.48^{b-d}	0.87^{e}	0.43^{gh}	1.95^{fg-i}	0.32^{e-g}	190.6^{ef}
$Z_0B_1M_1$	7.82^{b-d}	0.69^{e}	0.47^{f-h}	1.47^{g-i}	0.24^{gh}	211.4^{e}
$Z_0B_2M_0$	17.71^{a}	0.66^{e}	0.48^{f-h}	2.47^{f-i}	0.22^{h}	207.4^{e}
$Z_0B_2M_1$	5.31^{b-d}	0.65^{e}	0.41^{hi}	3.36^{ef}	0.22^{h}	177.6^{f}
$Z_1B_0M_0$	9.68^{bc}	0.68^{e}	0.41^{hi}	3.10^{e-g}	0.21^{h}	110.1^{gh}
$Z_1B_0M_1$	7.87^{b-d}	0.72^{e}	0.33^{i}	1.93^{f-i}	0.19^{h}	106.1^{gh}
$Z_1B_1M_0$	7.43^{b-d}	2.55^{ab}	0.61^{cd}	2.17^{f-i}	0.71^{a}	124.0^{g}
$Z_1B_1M_1$	3.45^{cd}	2.24^{bc}	0.65^{c}	1.25^{hi}	0.61^{b}	102.3^{gh}
$Z_1B_2M_0$	12.25^{ab}	2.14^{bc}	0.59^{c-e}	0.99^{i}	0.47^{c}	94.58^{h}
$Z_1B_2M_1$	5.96^{b-d}	1.58^{cd}	0.57^{c-f}	4.32^{de}	0.33^{ef}	94.69^{h}
$Z_2B_0M_0$	10.06^{bc}	0.99^{de}	0.56^{c-f}	2.88^{e-h}	0.31^{e-g}	255.7^{cd}
$Z_2B_0M_1$	6.70^{b-d}	1.23^{de}	0.50^{e-h}	5.36^{b-d}	0.25^{f-h}	255.7^{cd}
$Z_2B_1M_0$	7.46^{b-d}	1.06^{de}	0.51^{d-h}	6.01^{bc}	0.21^{h}	281.3^{bc}
$Z_2B_1M_1$	7.65^{b-d}	1.12^{de}	0.47^{f-h}	6.52^{bc}	0.20^{h}	282.1^{b}
$Z_2B_2M_0$	2.30^{d}	3.06^{a}	0.95^{a}	8.58^{a}	0.36^{de}	348.9^{a}
$Z_2B_2M_1$	4.93cd	2.03^{bc}	0.80^{b}	6.93^{ab}	0.30^{e-g}	354.1^{a}

***Means with the same letters in each column show no significant difference at P≤0.05 of LSD test. Z_0: no use of zeolite, Z_1: use of zeolite 5% by weight of the pot, Z_2: use of zeolite 10% by weight of the pot, B_0: no use of biochar, B_1: use of biochar 7.5% by weight of the pot, B_2: use of biochar 15% by weight of the pot, M0: no use of mycorrhiza, and M_1: use of mycorrhiza.**

Root dry weight: The highest root dry weight (2.55 g) was related to non-consumption of zeolite, biochar 15%, and mycorrhiza treatment ($Z_0B_2M_1$) in lead-contaminated soils while the lowest root dry weight (0.29 g) was recorded in the treatment with zeolite 10%, non-consumption of biochar, and the use of mycorrhiza ($Z_2B_0M_1$) in the lead-contaminated soils (Table 2).

The interaction effects of mycorrhiza, biochar, and zeolite in ($Z_0B_2M_1$) treatment increased the dry weight of the *Amaranthus caudatus* L. plants by 22.01% compared to the control (Table 3). The treatment with biochar (15%) and mycorrhiza appears to have increased root dry weight, while zeolite had no positive effect on improving this trait. Haji Najafi *et al*. (2016) found that the effect of biochar on root dry weight was significant. Mycorrhiza can positively affect plant growth by making soil phosphorus available and creating better conditions in the root environment for the plant. Ortiz (1996) argued that increased growth in plants is a result of the positive effects of mycorrhiza on

Table 3. Percentage of changes in the *Amaranthus caudatus* L. traits compared to the control under the influence of zeolite, biochar, and mycorrhiza in lead-contaminated soil

Treatment combination	Traits					
	Plant height	Number of sub-branches per plant	Biological performance in plant	Root dry weight per plant	Lead in rhizosphere soil	Lead in the root
Treatment that produced maximum of a trait*	32.19	48.17	194.23	22.01	125.27	66.67
Z_1**	-36.99	-44.44	-55.47	-66.99	92.15	30.59
Z_2	-6.85	47.89	43.33	-11.01	68.14	40.82
B_1	2.74	-20.39	-77.30	-75.60	92.01	-33.25
B_2	-11.64	-8.33	126.02	-20.10	79.11	35.99
M_1	-7.53	-44.44	-13.99	-30.62	68.77	15.46

***They were: $Z_0B_2M_1$ for plant height and root dry weight per plant, $Z_2B_0M_0$ for number of sub-branches per plant, $Z_1B_2M_0$ for biological performance of the plant and lead contents of roots, $Z_1B_1M_1$ for lead contents of soil rhizosphere, $Z_0B_2M_0$ for lead contents of leaves, $Z_2B_2M_0$ for catalase, peroxidase, and superoxide dismutase activities, $Z_1B_1M_0$ for ascorbate peroxidase activity, and $Z_2B_2M_1$ or glutathione reductase activity. **Z_1: Use of zeolite 5% by weight of the pot soil, Z_2: use of zeolite 10% by weight of the pot soil, B_1: use of biochar 7.5% by weight of the pot soil, B_2: use of biochar 15% by weight of the pot soil, and M_1: use of mycorrhiza.**

Continued of table 3.

Treatment combination	Traits					
	Lead in leaf	Catalase	Peroxidase	superoxide dismutase	Ascorbate peroxidase	Glutathione reductase
Treatment that produced maximum of a trait*	161.21	148.78	75.93	63.43	69.05	46.38
Z_1**	42.77	-44.72	-24.07	-40.95	-50.00	-54.49
Z_2	48.38	-19.51	3.70	-45.14	-26.19	5.71
B_1	39.82	-29.27	-20.37	-62.86	-23.81	-21.21
B_2	161.21	-46.34	-11.11	-52.95	-47.62	-14.26
M_1	52.95	-11.38	-7.41	-36.38	-14.29	-19.26

***They were: $Z_0B_2M_1$ for plant height and root dry weight per plant, $Z_2B_0M_0$ for number of sub-branches per plant, $Z_1B_2M_0$ for biological performance of the plant and lead contents of roots, $Z_1B_1M_1$ for lead contents of soil rhizosphere, $Z_0B_2M_0$ for lead contents of leaves, $Z_2B_2M_0$ for catalase, peroxidase, and superoxide dismutase activities, $Z_1B_1M_0$ for ascorbate peroxidase activity, and $Z_2B_2M_1$ for glutathione reductase activity. **Z_1: Use of zeolite 5% by weight of the pot soil, Z_2: use of zeolite 10% by weight of the pot soil, B_1: use of biochar 7.5% by weight of the pot soil, B_2: use of biochar 15% by weight of the pot soil, and M_1: use of mycorrhiza.**

Table 4. Pearson correlation coefficients between the measured traits of *Amaranthus caudatus* L. in lead contaminated soil

Traits	1- Plant height	2- Number of sub-branches per plant	3- Biological performance in plant	4- Root dry weight per plant	5- Lead in rhizosphere soil	6- Lead in the root	7- Lead in leaf	8- Catalase	9- Peroxidase	10- Superoxide dismutase	11- Ascorbate peroxidase
1											
2	0.33*										
3	0.67**	0.36**									
4	0.57**	0.42**	0.74**								
5	0.39**	0.12	0.28*	0.07							
6	0.09	0.21	0.41**	0.29*	0.27						
7	0.08	0.20	0.31*	0.13	0.26	0.25					
8	-0.04	-0.11	0.02	0.04	-0.02	-0.04	-0.29*				
9	-0.09	0.11	0.01	0.12	-0.02	-0.03	-0.27	0.79**			
10	-0.33*	-0.01	-0.26	-0.02	-0.45**	-0.28*	-0.34*	0.27*	0.48**		
11	0.16	-0.003	0.11	0.13	0.2	-0.09	-0.13	0.65**	0.44**	-0.26	
12	-0.31*	0.26	-0.22	0.11	-0.4**	-0.17	-0.17	0.12	0.45**	0.75**	-0.31*

ns, *, and ** show non-significant, significant at P≤0.05, and significant at P≤0.01, respectively.

increasing root absorption surface through penetration of fungus mycelium in soil and the host plant's access to greater volume of soil for transferring water and food to aerial organs. The effect of mycorrhiza species in plants' resistance to stress and their increased growth is discussed in terms of their capacity to protect plants and the adaptability of the fungus to the host plant (Rabie and Almadini, 2005). Among biological fertilizers, glomeromicota fungi, which are capable of symbiosis with the root of most crops, can help improve the growth and absorption of nutrients by plants through forming a growth hormone and enhancing resistance to soil diseases in plants (Heidari, 2009). A positive, strong, significant correlation ($0.6\leq r\leq 0.8$) was found between root dry weight and biological performance of the plant; also, the correlation between root dry weight and plant height and sub-branch number was positive and moderately significant ($0.4<r\leq 0.6$), and there was a positive and weakly significant correlation between root dry weight and the lead contents of roots ($0.4<r\leq 0.6$) (Table 4).

Phytoremediation potential, lead contents of the soil rhizosphere: The highest level of lead in the soil around the roots (rhizosphere) (49.92 µg/g) was related to the treatment with zeolite 5%, biochar 7.5%, and the use of mycorrhiza ($Z_1B_1M_1$) in lead-contaminated soil while the lowest concentration (22.16 µg/g) was recorded in the treatment composed of non-application of zeolite, biochar, and mycorrhiza ($Z_0B_0M_0$) in lead-contaminated soil (Table 2). The interaction effect of mycorrhiza, biochar, and zeolite in ($Z_1B_1M_1$) treatment increased the lead contents of the soil rhizosphere by 125.27% compared to the control (Table 3). Consumption of zeolite (5%), biochar (7.5%), and mycorrhiza seems to have increased concentration of lead in the rhizosphere. Owing to its high porosity, zeolites act as a superabsorbent and through preserving water and ion nutrients, improving their uptake, and preventing their loss can help in ionic balance and prevent the absorption of heavy metals (Khodarahmi *et al*., 2019). Inoculation of the plants with mycorrhiza reduces the absorption of heavy metals by increasing the contact surface of the root and soil. Also, by secreting organic acids and producing carbon dioxide, the fungus acidifies the root zone, which increases the absorption of phosphorus and infrequent elements and reduces harmful effects of heavy metals on the plant by keeping them in its hyphae (Nemati *et al.*, 2015; Gupta *et al*., 2002). A positive, weakly significant correlation ($0.2<r\leq 0.4$) was found between the concentration of lead in rhizosphere soil and the plant height and biological performance (Table 4).

Lead contents of the roots: The highest amount of lead in the root (20.05 µg/g) was related to the treatment with zeolite 5%, biochar 15%, and no mycorrhiza ($Z_1B_2M_0$) in lead-contaminated soil while the lowest concentration (8.03 µg/g) was related to the treatment with no consumption of zeolite, biochar 7.5% and non-application of mycorrhiza ($Z_0B_1M_0$) in lead-contaminated soil (Table 2). An increase by 67.66% in lead contents of roots was resulted from the interaction effect of mycorrhiza, biochar, and zeolite in ($Z_1B_2M_0$) treatment in comparison with the control (Table 3). The use of biochar (15% by weight) and zeolite (5% by weight) seems to have caused the absorption of lead in the roots, but the use of mycorrhiza had no effect on this trait. In an experiment conducted on Acacia Victoria plant, Kharmandar and Mahdavi (2016) found that the bioaccumulation factor, transfer factor, enrichment factor, and absorption index were higher in roots than in the stem. In fact, by accumulating cadmium in its roots, Acacia Victoria can prevent the transfer of this heavy metal to the aerial parts and the occurrence of toxicity in the plant (Kharmandar and Mahdavi, 2016).

Biochar increases the uptake of heavy metals in roots due to having oxygen containing functional groups and a wider specific surface compared to the primary raw material (Ali *et al*., 2014; Kabiri *et al*., 2021). Adewole *et al*. (2010) investigating the effects of mycorrhiza fungi on phytoremediation potential of sunflower plants in soils contaminated with lead and cadmium, showed that mycorrhiza reduced cadmium and lead uptake in roots and improved the plants' resistance to contamination. The results of the research by Esmaeilpour Fard *et al*. (2015) showed that the soil contaminated with a mixture of heavy metals and sepiolite increased the uptake of heavy metals by plants.

On the other hand, the results obtained in this study are not consistent with the findings of a number of studies reported in the literature. Haidarpour Sarami *et al*. (2022) showed that applying zeolite and mycorrhiza reduced the accumulation of lead in roots and shoots of corn and soybean plants. Also, Zheng *et al*. (2013) reported that the treatment of heavy metal-contaminated soils with biochar obtained from rice straw significantly decreased the concentration of zinc, cadmium, and lead compared to the control. In another study, Puga *et al*. (2015) observed that the application of sugarcane residue biochar in contaminated soils caused a decrease in the concentration of cadmium, lead, and zinc available to the plant compared to the control. Finally, Jafari *et al*. (2020) showed that application of biochar reduced the uptake of lead in sunflower roots compared to the control.

A positive, moderately significant correlation ($0.2<r\leq 0.4$) was found between the lead contents of roots and the biological yield of the plant. Also, the correlation between lead contents of roots and the biological yield of the plants was positive and weakly significant ($0.2<r\leq 0.4$) (Table 4).

Lead contents of the leaves: The highest amount of lead in leaves (17.71 µg/g) was found in the plants treated with zero zeolite, biochar 15%, and non-application of mycorrhiza ($Z_0B_2M_0$) in lead-contaminated soil. On the other hand, the lowest lead content (2.30 µg/g) was recorded in the treatment with zeolite 10%, biochar 15%, and no mycorrhiza ($Z_2B_2M_0$) in the soil contaminated with lead (Table 2).

Analysis of the interaction effect of mycorrhiza, biochar, and zeolite in ($Z_0B_2M_0$) treatment showed an increase in concentration of lead in leaves of cultivated amaranth plants by 161.21% compared to the control (Table 3). Application of biochar (15% by weight) seems to have caused the highest amount of lead uptake in the leaves, while zeolite and mycorrhiza had no effect. The results of Haji Najafi *et al*. (2016) showed that the effects of biochar on dry weight of shoots and roots and lead concentration in stem were significant ($P\leq0.01$) and it had a significant effect on lead concentrations of roots and leaves at $P\leq0.5$ probability level. In addition, the results of Moslehi *et al*. (2019) showed that the combined application of EDDS and vermicompost was the best modification for remediation of lead in plants. There was a positive, weakly significant correlation ($0.2<r\leq0.4$) between the amount of lead in leaves and the biological yield of the plants under study (Table 4).

Antioxidant enzymes, catalase: The highest activity of catalase enzyme (3.06 U mg^{-1}protein) belonged to the treatment with zeolite 10%, biochar 15% by, and non-application of mycorrhiza ($Z_2B_2M_0$) in lead-contaminated soil. The lowest activity of catalase (0.65 U mg^{-1}protein) was obtained from the treatment including non-application of zeolite, biochar 15%, and using mycorrhiza ($Z_0B_2M_1$) in lead-contaminated soil (Table 2). The interaction mycorrhiza, biochar, and zeolite treatments ($Z_2B_2M_0$) increased the activity of catalase enzyme by 148.78% in the cultivated amaranth plants compared to the control (Table 3). Applying zeolite (10%) and biochar (15%) seems to have increased the activity of catalase enzyme, while the use of mycorrhiza did not have any effect on improving the activity of this enzyme.

Catalase is one of the most important hydrogen peroxide scavenging enzymes. Increasing the activity of this enzyme increases the plant's resistance in stressful conditions and as a result, increases the yield. In stress conditions, the level of active oxygen species increases, and this is followed by an increase in the activity of antioxidant enzymes (Chavoushi *et al*., 2019). In their study, Teimouri *et al.* (2020) found huge differences in the activity of catalase among wheat cultivars under drought stress condition (cessation of irrigation at the end of the growing season), decreasing by 42% in some cultivars while increasing by 466% in others. Changes in catalase activity are dependent on the plant species, developmental stage, location of plant metabolism, and duration and intensity of stress (Mane *et al*., 2011). Similar results were reported by Farhadi *et al*. (2017) in corn plants.

A strong significant positive correlation ($0.6<r\leq0.8$) was found between catalase activity and that of peroxidase and ascorbate peroxidase enzymes. Also, the correlation between catalase and superoxide dismutase enzyme activity was positive and weakly significant ($0.2<r\leq0.4$) (Table 4).

Peroxidase: The highest activity of peroxidase (0.85 U mg^{-1} protein) was related to the treatment with zeolite 10%, biochar 15%, non-application of mycorrhiza ($Z_2B_2M_0$) in lead-contaminated soil. The lowest level of peroxidase activity (0.33 U mg^{-1}protein) was found in the plants treated with zeolite 5%, non-application of biochar, and mycorrhiza consumption ($Z_1B_0M_1$) in lead-contaminated soil (Table 2). The interaction of mycorrhiza, biochar, and zeolite in ($Z_2B_2M_0$) treatment increased the activity of peroxidase enzyme by 75.93% in the cultivated amaranth plants compared to the control (Table 3). Treatment with zeolite (10%) and biochar (15%) increased peroxidase activity, while the use of mycorrhiza had no effect on improving the activity of this enzyme.

The activity of peroxidase enzyme is easily detected throughout the life of different plants from the initial stages of germination to the senescence stage by controlling cell elongation, defence mechanisms, and several other functions (Niroomand *et al*., 2018). There are many reports on the increased level of peroxidase activity under abiotic stress conditions in different plants, e.g., sunflower (Gunes *et al*., 2008). In wheat cultivars, drought stress (withdrawing irrigation at the end of the growing season) resulted in increased activity of peroxidase (Teimouri *et al*., 2020). In their study on sunflower, Taher *et al*. (2018) found an increase in the activity of antioxidant enzymes under salinity stress, which can be attributed to the increased expression of the genes encoding the peroxidase enzyme or the stability of the protein molecules of this enzyme under oxidative damage as a result of the increase in cell age. Peroxidase has a key role in protecting the plant against stress by detoxifying hydrogen peroxide and removing malondialdehyde (Niroomand *et al*., 2018). Increased concentration of cadmium in the environment was found to decrease dry weight, pigment contents, absorption of zinc, and activity of peroxidase (Farjad Tehrani *et al*., 2023). Cadmium indirectly produces ROS, which in turn increase the activity of antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase. Studies have shown that depending on the type of plant species, the plant tissue under study, and metal concentration, cadmium can inhibit or stimulate the activity of a number of antioxidant enzymes (Chang *et al*., 2012; Shetty *et al*., 2012; Zheng *et al*., 2010). A strong significant positive correlation ($0.6<r\leq0.8$) was found between peroxidase and catalase enzyme activity. Also, the correlations between peroxidase enzyme and superoxide dismutase, ascorbate peroxidase, and glutathione reductase enzymes were positive and moderately significant ($0.4<r\leq0.6$) (Table 4).

Superoxide dismutase: The highest activity of superoxide dismutase enzyme (8.58 U mg^{-1} protein) was recorded in the plants treated with zeolite 10%, biochar 15%, and non-application of mycorrhiza ($Z_2B_2M_0$) in lead-contaminated soil; on the other hand, the lowest activity of this enzyme (0.99 U mg^{-1}protein) was related to the treatment with zeolite 5%, biochar 15%, and non-application of mycorrhiza ($Z_1B_2M_0$) in lead-

contaminated soil (Table 2). The activity of superoxide dismutase enzyme in cultivated amaranth plants increased by 63.43% under interaction effect of mycorrhiza, biochar, and zeolite ($Z_2B_2M_0$) compared to the control (Table 3). Applying zeolite (10%) and biochar (15%) increased the activity of superoxide dismutase enzyme. Superoxide radicals produced by the activation of the superoxide dismutase enzyme are converted into hydrogen peroxide, and the activity of the catalase enzyme prevents the accumulation of hydrogen peroxide. Since lead increases the production of reactive oxygen species (ROS) in plants and induces oxidative stress in them, an increase in the activity of certain antioxidant enzymes is observed in the plants (Darvishi and Kamajian, 2014). Research has shown a strong relationship between tolerance to oxidative stress caused by environmental stress, including heavy metal stress, and the increase in the concentration of antioxidant enzymes in photosynthetic plants. Since lead increases the concentration of ROS in plants and leads to the generation of oxidative stress in them, the activity of antioxidant enzymes, e.g., superoxide dismutase, peroxidase, ascorbate peroxidase, and glutathione reductase increases in their roots and leaves (Sharma and Dubey, 2005). Haidarpour Saremi *et al*. (2022) in their study on soybean and corn plants showed that the zeolite treatment decreased the activity of antioxidant enzymes while inoculation of the plants with mycorrhiza increased the effects of zeolite.

A strong significant positive correlation ($0.6<r\leq0.8$) was observed between activities of superoxide dismutase and glutathione reductase enzymes. Also, a moderately significant positive correlation ($0.4<r\leq0.6$) was found between the activities of superoxide dismutase and peroxidase enzyme, and the correlation between superoxide dismutase and catalase enzyme activities was weakly significant positive ($0.2<r\leq0.4$) (Table 4).

Ascorbate peroxidase: The highest activity of ascorbate peroxidase enzyme (0.71% U mg^{-1} protein) was recorded in the plants treated with zeolite (5%), biochar (7.5%), and without application of mycorrhiza ($Z_1B_1M_0$) in lead-contaminated soil, and the lowest activity of this enzyme (0.19 U mg^{-1} protein) was related to the treatment with zeolite (5% by weight), non-use of biochar, and use of mycorrhiza ($Z_1B_0M_1$) in lead-contaminated soil (Table 2). The interaction of mycorrhiza, biochar, and zeolite ($Z_1B_1M_0$) treatment increased the activity of ascorbate peroxidase enzyme by 69.05% compared to the control (Table 3). Applying zeolite (5%) and biochar (7.5%) increased the activity of ascorbate peroxidase enzyme, while mycorrhiza did not have any effect on improving the activity of this enzyme.

Enzymatic activities and the content of some compounds in plants are a common criterion to get an insight of stress conditions and the plant growth and development. The activity of antioxidant enzymes in plant cells increases in response to abiotic stress. Research has shown that there is a strong relationship between tolerance to oxidative stress induced by heavy metal and the concentration of antioxidant enzymes in photosynthetic plants (Darvishi and Kamajian, 2014). Ascorbate peroxidases play a key role in scavenging reactive oxygen species and protecting cells against their destructive effects in algae and plants. This enzyme has also an important role in the activity of the stomata by regulating the concentration of H_2O_2 in plant cells under salt stress, since the concentration of hydrogen peroxide acts as an important signal in moving the cells that protect the stomata (Montazerinezhad *et al*., 2013). Rahimizadeh *et al*. (2007) in their study on sunflower found an increase from 11% to 31% in the concentration of superoxide dismutase, catalase, and glutathione peroxidase antioxidant enzymes under high moisture stress condition and from 48% to 89% in the treatment with micronutrients (iron, manganese, zinc, and copper). Studies suggest a strong relationship between the plant's resistance to drought stress and antioxidant activities related to ascorbate (Laxa *et al*., 2019). Also, salinity was reported to increase electrolyte leakage while decreasing the activity of superoxide dismutase, ascorbate peroxidase, and catalase enzymes in sunflowers (Conceicao *et al*., 2019). The study by Gharehbaghli and Sepehri (2022) suggested that 10^{-5} M lead increased the activity of ascorbate peroxidase enzyme compared to the control, while a noticeable decrease in the activity of this enzyme was observed under severe lead stress (10^{-3} M). Ali *et al*. (2014) reported similar results with rapeseed roots, where increases and decreases were observed in the activity of ascorbate peroxidase under low and high levels of lead stress.

The correlation between ascorbate peroxidase and catalase enzymes was strong, significant, and positive ($0.6<r\leq0.8$). Also, a moderate and significant positive correlation ($0.4<r\leq0.6$) was found between ascorbate peroxidase and peroxidase enzymes (Table 4).

Glutathione reductase: The highest activity of glutathione reductase (354.1 U mg^{-1} protein), was recorded in the plants treated with zeolite (10%), biochar (15%), and mycorrhiza consumption ($Z_2B_2M_1$) while the lowest activity (94.58 U mg^{-1} protein) was related to the treatment with zeolite (5%), biochar (15%), and non-application of mycorrhiza ($Z_1B_2M_0$) in lead-contaminated soil (Table 2). The combined treatment of mycorrhiza, biochar, and zeolite ($Z_2B_2M_1$) increased the activity of glutathione reductase enzyme by 46.38% in cultivated amaranth plants in comparison with the control (Table 3). Treatment with zeolite (10%), biochar (15%), and mycorrhiza increased the activity of glutathione reductase enzyme, and glutathione reductase activity decreased in the absence of these treatments, especially in non-consumption of mycorrhiza. Mycorrhizal fungi, as one of the most important micro-organisms in soil, improves the absorption of water and nutrients in the host plants, thus indirectly reducing the effects of abiotic stresses by

establishing symbiosis with a wide range of plants in the three forms of ectomycorrhizal, endomycorrhizae, and ectendomycorrhizas symbiosis (Aghababaei and Reisi, 2011). Studies conducted by different researchers have shown that arbuscular mycorrhizal fungi (AMF) has the ability to improve the synthesis of antioxidants and increase their activity under heavy metal stress. For example, Cornejo *et al*. (2013) reported that AMF regulates the antioxidant activity and reduces the production of reactive oxygen species and their resulting stress in the host plant Cajanus cajan. Metal helps Yang *et al*. (2015) observed that the symbiotic relationship between AMF and *Robinia pseudoacacia* improves ROS scavenging potentials of the plants treated with different concentrations of lead and increases their enzyme activities. It was also reported that AMF stimulates the activation of antioxidant system in plants under cadmium stress (Cui *et al*., 2019). A strong significant positive correlation was found between glutathione reductase and superoxide dismutase enzymes ($0.6<r\leq0.8$) and the relationship between glutathione reductase and peroxidase enzymes was positive and moderately significant ($0.4<r\leq0.6$) (Table 4).

Conclusion

The maximum biological yield of the plant (54.05 g) and the highest concentration of lead in roots (20.05 mg/kg) were observed in the combined treatment of zeolite 5%, biochar 15%, and no mycorrhiza ($Z_1B_2M_0$). This is the best treatment for phytoremediation, in which the plants absorbed the highest concentration of lead. On the other hand, the highest accumulation of lead in leaves (17.71 mg/kg) was obtained in the treatment with no zeolite and mycorrhiza + using biochar 15% ($Z_0B_2M_0$). In view of the high biological performance of *Amaranthus caudatus* L. in this treatment (41.55 g), it is considered as one of the most effective plants in phytoremediation. According to the results, *Amaranthus caudatus* L. is recommended as an effective plant in the remediation of lead-contaminated soils, and the use of biochar can increase its phytoremediation potential.

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