Arbuscular Mycorrhiza Alters Metal Uptake and the Physio-biochemical Responses of \emph{Glycyrrhiza glabra} in a Lead Contaminated Soil

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Abstract
Arbuscular mycorrhizal (AM) fungi can affect the host’s ability to cope with several environmental stresses, such as heavy metal stress. Therefore, an experiment was conducted to assess the effect of the \emph{Funneliformis mosseae} inoculation on growth and physio-biochemical parameters and lead (Pb) accumulation in liquorice (\emph{Glycyrrhiza glabra} L.) under Pb stress. A factorial experiment was performed with the combination of two factors, fungi (inoculated and non-inoculated (NM)) and soil Pb levels (0, 150, 300, and 450 mg kg⁻¹ soil) with four replicates. In the presence of Pb, symbiosis with \emph{F. mosseae} exert positive effect on growth parameters, which was more significant in shoots than roots. Mycorrhization improved fresh and dry weights and length in shoot by 147, 112.5 and 83%, respectively, compared to NM plants at Pb150 level. Moreover, \emph{F. mosseae} significantly increased tolerance index and the concentrations of soluble sugars and flavonoids in shoots and proline, phosphorus, potassium, calcium, zinc and manganese in shoots and roots but decreased their malondialdehyde concentrations under Pb stress. The Pb concentrations, transfer and bioaccumulation factors of mycorrhizal plants were less than non-mycorrhizal ones. A positive correlation was also observed between glomalin secretion and colonization rate in Pb treated soils. These results indicate the importance of mycorrhizal colonization in alleviating the Pb-induced stress in liquorice, mainly through improving the nutrition, modifying reactive oxygen species detoxifying metabolites and reducing the translocation of Pb to shoots. Observations revealed that mycorrhization of liquorice would be an efficient strategy to use in the phytoremediation practices of Pb-contaminated soils.

Keywords \emph{Glycyrrhiza glabra} · Pb · \emph{Funneliformis mosseae} · Physio-biochemical parameters

Introduction
\emph{Glycyrrhiza glabra} L. (liquorice) is one of the most important medicinal plants in the world. Liquorice is a perennial and grass herb of the Fabaceae family, with a height of about 1.8–2.1 m with an extensive root system. The most important triterpene saponin in liquorice root is glycyrrhizin (up to 15%), which has different uses in food, industrial, cosmetic, and pharmaceutical industries (Öztürk et al. 2017). The anti-bacterial, anti-inflammatory, molluscicidal, insecticidal, antiulcer, and anti-allergic properties, as well as the activation of the immune system of glycyrrhizin, have been reported (Hosseini et al. 2018). The antivirus activity of glycyrrhizin against some viruses, coronavirus has been mentioned (Fuzimoto and Isidoro 2020). Therefore, food, medicinal and industrial uses of liquorice attract the researchers’ attention worldwide.

High concentrations of heavy metals (HMs) like chromium (Cr), nickel (Ni), lead (Pb), zinc (Zn), and thallium (Tl) in the environment have the harmful effects on many organisms. More concentrations of HMs in the biosphere result from increased human activities such as mining, smelting, combustion of fossil fuels, etc. (Singh et al. 2015). The HMs contaminated soils affect the quality and quantity of agricultural and food products (Janeeshma and Puthur 2020). Notably, physico-chemical properties and concentration HMs in soil, edaphic factors, redox reactions, and plant species affect toxicity and availability of HMs to the plants (Ghori et al. 2019).

Among bioremediation processes, use of microorganisms like arbuscular mycorrhizae (AM) has gained importance in the recent years. The application of AM fungi is
a cost-effective and environment friendly strategy that reduces HM phytotoxicity (Raiz et al. 2021). AM fungi, belonging to the phylum Glomeromycota, are ubiquitous rhizosphere microflora forming a symbiosis with more than 80% of terrestrial plants (Kumar 2018). The phylum Glomeromycota is divided into four orders: Glomerales, Diversisporales, Archaeosporales, Paraglomerales. *Funneliformis mosseae* is one of the most important AM fungi, which belongs to the order of Glomerales and the family of Glomeraeaceae (Kumar 2018; Redecker and Raab 2006; Schüaller et al. 2001). By enhancing the absorption of water and mineral nutrients, AM fungi improve the plant yield also the ability of the plant to overcome many environmental stresses such as salinity, nutrient imbalances, drought, HM toxicity, etc. (Fusconi and Berta 2012). The mechanisms involved in plants tolerance to HMs by AM fungi contain the increase of shoot biomass and antioxidants, the changes of root structure, and HM immobilization through the hyphal wall and glomalin exudation (Janeeshma and Puthur 2020). It should be noted that the effect of AM fungi on plant tolerance depends on the fungal isolate, HM and plant species (Ferrol et al. 2016).

There are few reports about the effect of HMs on liquorice. Bernard et al. (2008) investigated the tolerance of two liquorice varieties to 200 µM cadmium chloride *F. mosseae*. The phylum Glomeromycota is divided into four orders: Glomerales, Diversisporales, Archaeosporales, Paraglomerales. *Funneliformis mosseae* is one of the most important AM fungi, which belongs to the order of Glomerales and the family of Glomeraeaceae (Kumar 2018; Redecker and Raab 2006; Schüaller et al. 2001). By enhancing the absorption of water and mineral nutrients, AM fungi improve the plant yield also the ability of the plant to overcome many environmental stresses such as salinity, nutrient imbalances, drought, HM toxicity, etc. (Fusconi and Berta 2012). The mechanisms involved in plants tolerance to HMs by AM fungi contain the increase of shoot biomass and antioxidants, the changes of root structure, and HM immobilization through the hyphal wall and glomalin exudation (Janeeshma and Puthur 2020). It should be noted that the effect of AM fungi on plant tolerance depends on the fungal isolate, HM and plant species (Ferrol et al. 2016).

There are few reports about the effect of HMs on liquorice. Bernard et al. (2008) investigated the tolerance of two liquorice varieties to 200 µM cadmium chloride (CdCl₂) solution and the accumulation of this toxic HM both *in vitro* and *in vivo* conditions and their results showed that plantlets and callus tissues of liquorice were able to accumulate very high amounts of Cd without a lot of damage and this plant may be consider as hyperaccumulator of Cd. Also, the positive effect of inoculation with AM fungi on the growth parameters of two ecotypes of liquorice under Pb and Cd stress has been studied and both *Ramjerd* and *Baft* ecotypes introduced as efficient species for stabilization of Cd and Pb (Tabrizi et al. 2021). A recent report has been suggested liquorice root as an effective and cheap bioabsorbent for copper ions from wastewater (Pirsalami et al. 2021).

Exposure of a plant to single stress activates the plant response, which facilitates tolerance to different types of stresses. This phenomenon is known as cross-tolerance (Foyer et al. 2016). Therefore, salt tolerant plants might resist other environmental stresses such as HM stress. It seems metal tolerance mechanisms rely on the same physiological mechanisms involved in salt tolerance to some extent. Understanding of the phenomenon of cross-tolerance of salinity with HMs stress in salt tolerant plants could very well boost their potential use in phytoremediation (Nikalje and Suprasanna 2018). The heavy metal phytoremediation capacity of salt tolerant species from different plant families has been reported in the literature (Manousaki and Kalogeras 2011; Nikalje and Suprasanna 2018; García Caparrós et al. 2022). Also, liquorice has been reported as a salt-tolerant plant, significantly to moderate salinity (4–8 dS m⁻¹) of soil (Amanifar et al. 2019). Therefore, the use of such species together with the mycorrhizal association in phytoremediation projects could be an alternative to remediation of HMs polluted soils.

The objective of this study was to evaluate the tolerance threshold of liquorice for phytoremediation and also the effect of symbiosis with *F. mosseae* on Pb detoxification and plant tolerance. We hypothesized that AM-mediated Pb tolerance mainly is attributed to the changes of physio-biochemical parameters and accumulation of Pb in liquorice.

**Material and Methods**

**Preparation of Mycorrhizal Fungi Inoculum**

*F. mosseae* soil-based inoculum was prepared by the Laboratory of Soil Biology at the University of Shiraz, Iran. This mycorrhizal inoculant was initially isolated from Anghran Zinc and lead Mine in Zanjan Province, Iran (Zarei et al. 2008). The propagation of AM fungus spores was performed using *Zea mays* L. as the trap plant in a combination of sterilized sand and soil (1:1 V/V). The mycorrhizal inoculum contained the substratum, root fragments (with root colonization rate of 87%), spores (average number of spores was 43 spores per gram of inoculum), and mycelium.

**Plant Material and Treatment Conditions**

An experiment was performed in a factorial randomized complete design with a combination of two factors, fungi (inoculated and non-inoculated) and soil Pb levels (0, 150, 300, and 450 mg Kg⁻¹ soil) with four replicates. The Pb levels were selected according to the relevant reports (Jamal et al. 2019; Tabrizi et al. 2021) and our preliminary experimental results in laboratory. This experiment was performed in a plant growth chamber at the Department of Biology, University of Zanjan, Zanjan, Iran, during 2018 and 2019. Liquorice seeds prepared from the Company of Isfahan Pakan Bazr, were surface disinfected with 3% sodium hypochlorite for 3 min and rinsed three times with sterile, distilled water. Then half of the seeds were sown in seedling trays containing 200 g autoclaved soil plus 20 g of *F. mosseae* soil-based inoculum for mycorrhizal treatments, while the other half of the seeds were sown in the same amount of sterilized soil plus 20 g of autoclaved soil-based inoculum for non-mycorrhizal treatments. The inoculums filtrate was added in all treatments to establish a uniform bacterial composition. Seed germination was started 7 days after sowing, and 15 days later about 80% of seeds were germinated. Seedlings were grown in a growth chamber at 25–27 °C with 16 h photoperiod about two months and
misted regularly with distilled water until reaching the four-
true-leaves growth stage.

For Pb stress treatment, a sandy clay loam soil was col-
lected from the surface layer (0–20 cm depth) from the re-
search farm located at the University of Zanjan (36° 41’ N;
48° 30’ E). The properties of collected soil containing tex-
ture (Gee and Bauder 1986) sandy clay loam, available P
(Olsen and Sommers 1982) 8 ppm, NH4Ac extractable K
(Kundsen et al. 1982) 191 ppm, organic carbon (Walkley
and Black 1934) 0.93%, pH 7.93, electrical conductivity
(EC) (Rhoades and Oster 1986) 1.30 dS m–1, and field ca-
pacity (FC) (Cassel and Nielsen 1986) 18.6% (w/w) were
measured. The sieved autoclaved soil was artificially con-
taminated using salt of lead nitrate (Pb(NO3)2).

To achieve the Pb treatments, the required amounts of
dissolved Pb(NO3)2 in sterile distilled water to FC moisture
content were sprayed to all treatments. Also, considering
the highest level of Pb(NO3)2 applied to soil, the appropri-
ate amounts of ammonium nitrate (NH4NO3) were added
to all treatments except Pb450 to equalize nitrogen level.
Therefore, the required amounts of Pb(NO3)2 and NH4NO3
salts thoroughly dissolved in 2560 mL of sterile distilled
water. Then the prepared solution was added and mixed
into 16 kg of air-dried soil for each level of Pb treatment.
The contaminated soils with different Pb levels were in-
cubated in a greenhouse (temperature of 25 °C/20 °C) with
wet (maintain moisture at FC level) and dry periods for
two months. After incubation time, the content of avail-
able Pb was measured by the DTPA method (Lindsay and
Norvell 1978). The results are described in Table 1. Af-
after two months, the healthy mycorrhizal and non-mycorrhizal
liquorice (NM) seedlings (two-month-old) were transferred
to disinfected plastic pots (15 cm in diameter). Each pot
contained 2 kg of sterile soil with different levels of Pb and
four uniform plants. Then the plants were grown in a cham-
ber with a photoperiod 16 h (white fluorescent lamps; irra-
diance of 200 µmol/m2 s) and a temperature of about 25 to
27 °C and irrigated with distilled water to 80% FC moisture
content for each pot. After three months, the plants were
harvested from pots.

| Treatment | Available Pb |
|-----------|--------------|
| Control   | 4.52         |
| Pb150     | 92.64        |
| Pb300     | 165.72       |
| Pb450     | 269.62       |

**Growth Parameters and Mycorrhizal Colonization
Assessment**

First, the plants were taken out of the pot, and the growth
parameters of four plants in each pot, such as the length
of roots and shoots and fresh and dry weights of roots and
shoots, leaf area, and relative water content (RWC). After
drying plant samples at 70 °C for 48 h, dry weight was de-
termined. RWC was estimated according to Ünyayar et al.
(2004).

For mycorrhizal colonization assessment, root segments
(~1 cm length) after clearing using 10% KOH were stained
with 0.05% trypan blue in lactoglycerol (Phillips and Hay-
man 1970). Then the colonization rate for each treatment
was estimated by the gridline intersect method (Giovannetti
and Mosse 1980).

**Physio-biochemical Parameters**

**Photosynthetic Pigments**

For determination of the chlorophylls (Chl) and carotenoids
(Car) contents in leaves, 200 mg of plant tissue was ho-
mogenized in 80% acetone (v/v), then the absorbance of
the pigment extraction was read at wavelengths 647 nm
(Chl a), 663 nm (Chl b), and 470 nm (Car) using the spec-
trophotometer (HACH, DR 5000™ UV_Vis Spectropho-
tometer, USA). The amount of photosynthetic pigments
was calculated by the following equations (Lichtenthaler
and Buschmann 2001):

\[
\text{Chl a (µg mL}^{-1}\text{)} = (12.25 A663 - 2.79 A647) \\
\text{Chl b (µg mL}^{-1}\text{)} = (21.50 A647 - 5.10 A663) \\
\text{Total Chl (µg mL}^{-1}\text{)} = \text{Chl a + Chl b} \\
\text{Car (µg mL}^{-1}\text{)} = (1000 A470 - 1.82 \text{Chl a} - 85.02 \text{Chl b}) / 1981
\]

**Total Soluble Sugar**

The total soluble sugar content of the shoots and roots
was determined using Kabiri et al. (2014) method. Fresh
samples (0.05 g) were extracted using 2.5 mL 80% ethanol
(v/v) at 95 °C for 60 min. After extraction filtration, the
ethanol was evaporated and the resulting precipitate dis-
solved in 2.5 mL of distilled water. For assay of soluble
sugar, the mixture of 200 µL of extraction and 5 mL of an-
throne reagent (0.2% anthrone dissolved in sulfuric acid)
was placed in a boiling water bath for 17 min and then
cooled in a running tap-water bath. Finally, the mixture
absorbance was recorded at 625 nm, and the soluble sugar
content was measured using glucose as a standard.
Proline

For extraction of proline, fresh plant materials (0.05 g of the shoot and 0.02 g of the root) were homogenized in 10 mL of 3% sulfosalicylic acid (w/v) and filtered with Whatman filter paper.

For assay of proline, the mixture of 2 mL of the extraction, 2 mL of 2.5% acid-ninhydrin reagent, and 2 mL of glacial acetic acid was placed in a boiling water bath for 1 h. Then the mixture was cooled in an ice bath, and 4 mL of toluene was added. The absorbance of the upper phase was read at 520 nm, and the amount of proline was calculated using a standard curve of L-proline and reported as µmol g⁻¹ FW (Ullah et al. 2019).

Total Phenolics and Flavonoids

Total phenolics and flavonoids contents were extracted with pure methanol at room temperature for 48 h. After filtration of the extracts and evaporation of the methanol, the resulting precipitate was dissolved in 4 mL of distilled water or pure methanol. The aqueous (phenolics) and methanolic (flavonoid) extractions were used for analysis.

Total phenolics content of the aqueous extract was measured by a method involving Folin-Ciocalteu reagent and gallic acid (GAE) standard at 765 nm (Pourmorad et al. 2006). The amount of total phenolics was expressed as mg GAE equivalent g⁻¹ DW.

Flavonoid content was determined by Chang et al. (2002) using the aluminum chloride method at 415 nm. The standard curve was prepared at concentrations 0–200 µg mL⁻¹ quercetin and the flavonoid content was reported as mg quercetin equivalent g⁻¹ DW.

Malondialdehyde (MDA)

The MDA amount as a product of lipid peroxidation was measured according to Velikova et al. (2000). Fresh plant materials (0.15 g) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and then centrifuged (5 min, 10,000 × g). After adding 4 mL of thiobarbituric acid (TBA) reagent (0.5% (w/v) TBA dissolved in 20% (w/v) TCA) to 1 mL of the supernatant, the mixture was incubated in a hot water bath at 95 °C for 30 min and then immediately cooled and centrifuged (10 min, 10,000 × g). The supernatant absorbance was determined at 532 (A532: MDA-TBA complex) and 600 nm (A600: nonspecific pigments), which A532–A600 and extinction coefficient of 115 mM⁻¹ cm⁻¹ were used to calculate the MDA content as µmol g⁻¹ FW.

Mineral Nutrients and Pb Concentrations

For the extraction of Pb and mineral nutrients, 0.3 g of the dried sample was ground with a laboratory mill and digested using 6 mL of nitric acid. Concentrations of Pb and mineral nutrients such as potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), and manganese (Mn) were measured by Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES). Phosphorus concentration was determined spectrophotometrically using the vanadate-molybdate colorimetric method at 430 nm (Heidari and Karami 2014).

Phytoremediation Potential Evaluation

The bioaccumulation factor (BCF), translocation factor (TF), tolerance index (TI) were calculated by the following equations (Gabos et al. 2009):

\[
BCF = \frac{\text{Pb concentration in the shoot}}{\text{Pb concentration in soil}} \quad (5)
\]

\[
TF = \frac{\text{Pb concentration in the shoot}}{\text{Pb concentration in the root}} \quad (6)
\]

\[
TI = \frac{\text{Dry weight of the plant in contaminated soil}}{\text{Dry weight of the plant in control soil}} \quad (7)
\]

Easily Glomalin-related Soil Protein (EE-GRSP)

The EE-GRSP concentration was measured according to Wright and Upadhyaya’s method (1996). The extraction of EE-GRSP from 1 g soil (< 2 mm) was performed using 8 mL of 20 mM citrate buffer (pH 7.0) in an autoclave (121 °C, 60 min). After centrifugation of mixture (10,000× g 5 min), the absorbance of the supernatant was read at 595 nm using bovine serum albumin as a standard (Bradford 1976).

Statistical Analysis

The data determined in four replicates were analyzed by the two-way analysis of variance (ANOVA) using the SPSS 25.0 software. The comparison of means was performed according to Duncan’s Multiple Range Test (DMRT) at P < 0.05. The experiment was bifactorial (fungus× Pb) in a completely randomized design (CRD).

Results

Mycorrhizal Colonization

Pb stress significantly affected root colonization rate (P < 0.01), ranging from 41 to 66% (Table 2 and Fig. 1). Mycorrhizal colonization at Pb150 and Pb300 levels was significantly reduced compared to the control level, while...
### Table 2: Results of two-way ANOVA of measured parameters of *G. glabra* L. as affected using fungal inoculation and soil Pb levels

| Parameters                  | Fungi Stress | Fungi × Stress | Parameters                  | Fungi Stress | Fungi × Stress |
|-----------------------------|--------------|----------------|-----------------------------|--------------|----------------|
| Shoot dry weight            | $F_{1,24} = 135.439^{**}$ | $F_{3,24} = 1.310^*$ | Shoot malondialdehyde      | $F_{1,16} = 1.972^{ns}$ | $F_{3,16} = 4.390^*$ |
| Root dry weight             | $F_{1,24} = 35.807^{**}$   | $F_{3,24} = 25.260^{**}$ | Root malondialdehyde       | $F_{1,16} = 179.300^{**}$ | $F_{3,16} = 69.471^{**}$ |
| Plant dry weight            | $F_{1,24} = 145.191^{**}$ | $F_{3,24} = 36.918^{**}$ | Root soluble sugars        | $F_{1,16} = 4160.868^{**}$ | $F_{3,16} = 708.815^{**}$ |
| Shoot fresh weight          | $F_{1,24} = 373.779^{**}$ | $F_{3,24} = 2.677^{**}$   | Root soluble sugars        | $F_{1,16} = 52.690^{**}$   | $F_{3,16} = 75.402^{**}$ |
| Root fresh weight           | $F_{1,24} = 0.295^{ns}$   | $F_{3,24} = 19.827^{**}$   | Shoot phenolics           | $F_{1,16} = 24.599^{**}$   | $F_{3,16} = 260.923^{**}$ |
| Shoot length                | $F_{1,24} = 196.548^{**}$ | $F_{3,24} = 3.097^{**}$   | Root phenolics            | $F_{1,16} = 94.085^{**}$   | $F_{3,16} = 329.793^{**}$ |
| Root length                 | $F_{1,24} = 0.089^{ns}$   | $F_{3,24} = 5.834^{**}$   | Shoot flavonoids          | $F_{1,16} = 127.213^{**}$ | $F_{3,16} = 22.551^{**}$ |
| Leaf area                   | $F_{1,24} = 149.945^{**}$ | $F_{3,24} = 5.665^{**}$   | Root flavonoid            | $F_{1,16} = 181.841^{**}$ | $F_{3,16} = 479.930^{**}$ |
| RWC                         | $F_{1,24} = 22.391^{**}$ | $F_{3,24} = 16.812^{**}$ | EE-GRSP                   | $F_{1,16} = 19.431^{**}$   | $F_{3,16} = 31.514^{**}$ |
| R/S                         | $F_{1,24} = 235.448^{**}$ | $F_{3,24} = 130.098^{**}$ | Shoot P                   | $F_{1,16} = 107.220^{**}$ | $F_{3,16} = 22.347^{**}$ |
| Root colonization           | –                      | $F_{3,8} = 40.670^{**}$ | Root P                    | $F_{1,16} = 3.939^{**}$    | $F_{3,16} = 35.545^{**}$ |
| Chlorophyll a               | $F_{1,16} = 16.819^{**}$ | $F_{3,16} = 194.567^{**}$ | Shoot Pb                  | $F_{1,16} = 2894.341^{**}$ | $F_{3,16} = 7208.515^{**}$ |
| Chlorophyll b               | $F_{1,16} = 10.369^{**}$ | $F_{3,16} = 189.884^{**}$ | Root Pb                   | $F_{1,16} = 891.894^{**}$   | $F_{3,16} = 5703.672^{**}$ |
| Total Chlorophyll           | $F_{1,16} = 19.815^{**}$ | $F_{3,16} = 448.662^{**}$ | Tolerance Index           | $F_{1,12} = 66.423^{**}$   | $F_{2,12} = 120.676^{**}$ |
| Carotenoids                 | $F_{1,16} = 13.335^{**}$ | $F_{3,16} = 51.293^{**}$   | Bioaccumulation Factor     | $F_{1,16} = 1002.562^{**}$ | $F_{3,16} = 1299.478^{**}$ |
| Shoot proline               | $F_{1,16} = 5461.742^{**}$ | $F_{3,16} = 1374.151^{**}$ | Translocation Factor      | $F_{1,16} = 106.239^{**}$   | $F_{3,16} = 11.485^{**}$   |
| Root proline                | $F_{1,16} = 1142.537^{**}$ | $F_{3,16} = 354.756^{**}$ | –                         | –                         | –                         |

ns not significant, RWC relative water content, R/S ratio of dry weight root to shoot, EE-GRSP Easily glomalin-related soil protein, P phosphorus, Pb lead

*P < 0.05, **P < 0.01
Fig. 1  Mycorrhizal colonization rates of liquorice grown under different levels of Pb. Pb150, Pb300, and Pb450 were 150, 300, and 450 mg Pb Kg⁻¹ soil, respectively. Different levels indicate a significant difference (P<0.01) according to the Duncan’s test

root colonization at the Pb450 level was significantly higher than other Pb levels. Also, mycorrhizal colonization increased linearly with the soil Pb concentration (Fig. 1).

**Growth Characteristics**

The ANOVA of growth characteristics showed that Pb levels, mycorrhiza, and their interaction significantly affected dry weights of root and total plant, root/shoot ratio, length of shoot, leaf RWC, and leaf area. However, the fresh weight and length of the root by lead and mycorrhiza×Pb interaction, the dry weight of shoot by Pb and mycorrhiza, and the fresh weight of shoot by mycorrhiza and mycorrhiza×Pb interaction were significantly affected (Table 2).

Assessing the growth characteristics of liquorice under different Pb levels showed that the inoculation with *F. mosseae* improved shoot growth parameters such as fresh and dry weights, length, and leaf area at all Pb levels as the highest amount was related to AM plants at Pb150 level, which increased 147, 112.5, 83 and 133%, respectively, compared to NM plants (Table 3). Unlike the shoot, the highest amount of root biomass and length of NM plants were observed at Pb150 level, while inoculation with AM fungus caused a significant reduction at the same level (Table 3). Overall, the stimulating effect of AM fungus on the growth characteristics of the shoot was greater than the root. Inoculation with *F. mosseae* caused the improvement of 24, 53 and 15% in dry weight of the plants compared to the corresponding NM plants at levels of Pb150, Pb300 and Pb450, respectively. Moreover, the root/shoot ratio of NM plants in all Pb levels was significantly higher than AM plants (Table 3).

**Biochemical Parameters**

**Photosynthetic Pigments**

Mycorrhiza, Pb stress, and their interaction significantly affected the contents of Chl a, Chl b, total Chl, and carotenoid (P<0.01) (Table 2). In NM plants, a significant increase in Chl a, Chl b, and total Chl contents was observed during Pb stress. Fungal inoculation induced two different effects according to the lead level: a significant increase in Chl a, Chl b, and total Chl contents at the control and Pb300 levels and a significant decrease in their contents at Pb150 and Pb450 levels, although changes in Chl b content at high Pb levels (Pb300 and Pb450) were not significant (Table 4). In NM plants, Pb stress significantly increased the carotenoid content at Pb150 and Pb300 levels while decreasing it at the Pb450 level. Also, the effect of fungal inoculation on the carotenoid content at the control and Pb150 levels was like chlorophyll, but unlike chlorophyll, a significant increase with fungal inoculation was observed at the Pb450 level (Table 4). The *F. mosseae* fungus increased the contents of Chl a, Chl b and total Chl by 54.2, 66.8 and 68.3%, respectively at Pb control level but decreased them by 47.4, 47.8 and 46.7%, respectively at Pb150 level.

**Proline**

Pb stress, mycorrhizal inoculation, and their interaction influenced shoot and root proline content significantly (Table 2). In NM plants, the shoot and root proline contents at different Pb levels except for Pb300 were significantly higher than Pb control (Fig. 2a,b). The symbiosis with AM fungus significantly increased shoot and root proline contents at different Pb levels, and the highest content was observed at Pb450 level. Mycorrhization improved proline content in shoot and root by 212 and 235.5%, respectively, compared to NM plants at Pb450 (Fig. 2a,b).

**Total Soluble Sugars (TSS)**

Based on the data shown in Table 2, mycorrhiza, Pb stress, and mycorrhiza×Pb interaction significantly affected TSS amount in shoots and roots (P<0.01). Severe Pb stress significantly increased the shoot TTS content in comparison with the control level, but the difference between Pb300 and Pb450 levels was not significant (Fig. 2c). At different Pb levels, the shoot TSS content in the AM plants was significantly higher than their corresponding NM plants, and the highest amount was observed at the level of Pb300 (Fig. 2c).

The TSS content in roots of both NM and AM plants significantly decreased at the highest Pb level (Pb450) com-
Table 3 Effect of mycorrhizal inoculation and soil Pb levels on dry weights (DW), fresh weights (FW), root to shoot dry weight ratio (R/S), lengths (L), relative water content (RWC) and leaf area (LA) of G. glabra

| Pb stress | AM status | Shoot DWa (g pot−1) | Root DW (g pot−1) | Plant DW (g pot−1) | Shoot FW (g pot−1) | Root FW (g pot−1) | R/S | Shoot L (cm) | Root L (cm) | RWC | LA (mm²) |
|-----------|-----------|----------------------|-------------------|-------------------|-------------------|-------------------|-----|-------------|-------------|------|----------|
| Control   | NM        | 0.10 ± 0.001c        | 0.09 ± 0.005b     | 0.17 ± 0.007d     | 0.23 ± 0.007c     | 0.18 ± 0.006b     | 1.00 ± 0.015c | 16.35 ± 0.36c | 18.07 ± 0.585b | 68.46 ± 1.852b | 310.50 ± 31.993c |
| Pb150     | NM        | 0.08 ± 0.006d        | 0.13 ± 0.009a     | 0.21 ± 0.006c     | 0.19 ± 0.007d     | 0.29 ± 0.016e     | 1.43 ± 0.036a | 14.82 ± 0.437d | 21.97 ± 0.636a | 63.04 ± 0.283a | 278.25 ± 20.946d |
| Pb300     | NM        | 0.17 ± 0.001d        | 0.09 ± 0.004b     | 0.26 ± 0.004a     | 0.47 ± 0.033d     | 0.24 ± 0.005bc    | 0.51 ± 0.011f | 27.10 ± 0.673d | 19.50 ± 0.868b | 48.82 ± 1.022d | 648.25 ± 55.773d |
| Pb450     | NM        | 0.14 ± 0.009d        | 0.09 ± 0.002b     | 0.23 ± 0.007e     | 0.37 ± 0.014f     | 0.20 ± 0.031h     | 0.61 ± 0.003c | 22.62 ± 0.875b | 18.01 ± 0.604bc | 53.17 ± 1.421e | 592.50 ± 25.503a |
| Pb150     | Fm        | 0.14 ± 0.011b        | 0.04 ± 0.001b     | 0.20 ± 0.005c     | 0.39 ± 0.010b     | 0.16 ± 0.021d     | 0.30 ± 0.011f | 21.37 ± 0.976b | 20.19 ± 1.033b | 73.05 ± 0.906b | 422.25 ± 44.659d |
| Pb300     | Fm        | 0.17 ± 0.001d        | 0.09 ± 0.004b     | 0.26 ± 0.004a     | 0.47 ± 0.033d     | 0.24 ± 0.005bc    | 0.51 ± 0.011f | 27.10 ± 0.673d | 19.50 ± 0.868b | 48.82 ± 1.022d | 648.25 ± 55.773d |
| Pb450     | Fm        | 0.15 ± 0.008d        | 0.09 ± 0.006b     | 0.23 ± 0.007e     | 0.37 ± 0.014f     | 0.20 ± 0.031h     | 0.61 ± 0.003c | 22.62 ± 0.875b | 18.01 ± 0.604bc | 53.17 ± 1.421e | 592.50 ± 25.503a |

Data present the means of four replicates ± standard error. Values labeled with the different letters present significant differences (p < 0.05) according to the Duncan test. Pb150, Pb300, and Pb450 are 150, 300, and 450 mg Pb Kg−1 soil, respectively. NM and Fm are non-mycorrhizal and F. mosseae inoculated plants.

aThere were three plants in each pot.

Table 4 Effect of mycorrhizal inoculation and soil Pb levels on photosynthetic pigments of leaves, and total phenolics, flavonoids, malondialdehyde (MDA) contents in root and shoot of G. glabra

| Pb stress | AM status | Chlorophyll a (mg g−1FW) | Chlorophyll b (mg g−1FW) | Total Chlorophyll (mg g−1FW) | Carotenoids (mg g−1FW) | Shoot total phenolics (mg GAE g−1DW) | Root total phenolics (mg GAE g−1DW) | Shoot flavonoids (mg g−1DW) | Root flavonoids (mg g−1DW) | Shoot MDA (µmol g−1FW) | Root MDA (µmol g−1FW) |
|-----------|-----------|--------------------------|--------------------------|-------------------------------|-------------------------|--------------------------------------|-----------------------------------|----------------------------|--------------------------|--------------------------|--------------------------|
| Control   | NM        | 0.72 ± 0.051c            | 0.38 ± 0.008d            | 1.10 ± 0.0071                | 0.22 ± 0.0088           | 6.32 ± 0.070a                       | 2.76 ± 0.147c                     | 1.55 ± 0.014b               | 2.79 ± 0.174a             | 2.57 ± 0.078b             | 2.57 ± 0.078b             |
| Pb150     | NM        | 1.11 ± 0.007c            | 0.63 ± 0.004b            | 1.75 ± 0.008d                | 0.27 ± 0.002b           | 3.91 ± 0.214b                       | 2.99 ± 0.090b                     | 0.42 ± 0.010c               | 1.85 ± 0.160b             | 2.65 ± 0.099b             | 2.65 ± 0.099b             |
| Pb300     | NM        | 1.16 ± 0.023c            | 0.69 ± 0.014a            | 1.84 ± 0.029c                | 0.31 ± 0.0044           | 3.24 ± 0.055c                       | 0.92 ± 0.030f                     | 0.59 ± 0.013d               | 2.30 ± 0.181b             | 5.20 ± 0.074d             | 5.20 ± 0.074d             |
| Pb450     | NM        | 1.22 ± 0.0176            | 0.69 ± 0.002c            | 1.91 ± 0.012d                | 0.31 ± 0.014c           | 3.11 ± 0.214b                       | 3.63 ± 0.029h                     | 0.52 ± 0.006d               | 2.27 ± 0.146c             | 4.50 ± 0.196e             | 4.50 ± 0.196e             |
| Pb150     | Fm        | 0.61 ± 0.021d            | 0.36 ± 0.003d            | 0.98 ± 0.012c                | 0.14 ± 0.001c           | 2.33 ± 0.073d                       | 1.63 ± 0.032f                     | 0.46 ± 0.021f               | 2.63 ± 0.177b             | 2.55 ± 0.018b             | 2.55 ± 0.018b             |
| Pb300     | Fm        | 1.22 ± 0.0176            | 0.69 ± 0.002c            | 1.91 ± 0.012d                | 0.31 ± 0.014c           | 3.11 ± 0.214b                       | 3.63 ± 0.029h                     | 0.52 ± 0.006d               | 2.27 ± 0.146c             | 4.50 ± 0.196e             | 4.50 ± 0.196e             |
| Pb450     | Fm        | 0.61 ± 0.021d            | 0.36 ± 0.003d            | 0.98 ± 0.012c                | 0.14 ± 0.001c           | 2.33 ± 0.073d                       | 1.63 ± 0.032f                     | 0.46 ± 0.021f               | 2.63 ± 0.177b             | 2.55 ± 0.018b             | 2.55 ± 0.018b             |

Data present the means of four replicates ± standard error. Values labeled with the different letters present significant differences (p < 0.05) according to the Duncan test. Pb150, Pb300, and Pb450 are 150, 300, and 450 mg Pb Kg−1 soil, respectively. NM and AM are non-mycorrhizal and arbuscular mycorrhizal inoculated plants.
The contents of proline in shoot (a) and root (b), and contents of soluble sugars in shoot (c) and root (d) of *G. glabra* non-inoculated (NM) or inoculated with arbuscular mycorrhizal (AM) under different levels of Pb. Pb150, Pb300, and Pb450 are 150, 300, and 450 mg Pb Kg\(^{-1}\) soil, respectively. Error bars indicate standard deviation.

Inoculation with AM fungus significantly increased TSS content of root-only at Pb150 and Pb300 levels (Fig. 2d).

**Total Phenolics (TP) and Flavonoid**

Mycorrhiza, Pb stress, and mycorrhiza×lead interaction significantly affected shoots and roots TP and flavonoid contents (Table 2). Pb stress caused a significant decrease in the amounts of shoot TP and root flavonoid at all Pb levels. Shoot flavonoid was also decreased at Pb300 and Pb450 levels. While a considerable increase for root TP was observed at Pb300 and Pb450 compared to Pb control (Table 4). Fungal inoculation significantly decreased the shoot TP content at Pb control and Pb150 levels and increased it at Pb300 and Pb450 levels, but its effect on root TP was the opposite of shoot TP content. Also, *F. mosseae* inoculation significantly increased shoot flavonoid content at all Pb levels but significantly decreased root flavonoid content at all Pb levels except Pb300 (Table 4).

**MDA Content**

Lead stress and mycorrhiza×lead interaction significantly modified shoot and root MDA content in liquorice, but fungus affected only the root MDA content (Table 2). A significant increase in root MDA content was observed at Pb150, and Pb300 levels, and *F. mosseae* induced a significant reduction at the same Pb levels (Table 4). Also, the shoot MDA content in the AM plants was lower than their corresponding NM plants at levels of control and Pb450 by 35.7 and 42.8%, respectively (Table 4).

**Concentrations of Mineral Nutrients in Plant**

The shoots and roots P concentrations were significantly affected by Pb stress and mycorrhiza×Pb interaction (Table 2). P concentration in the shoot of NM plants at different Pb levels showed a significant decrease compared to the control, and inoculation with AM fungus significantly increased P concentration at all Pb levels (Fig. 3a).

Pb stress significantly decreased root P concentration of NM plants except for the Pb150 level, which showed a significant increase compared to the control. A 143 and 99% increase in root P concentration of AM plants was observed at Pb300 and Pb450 levels, respectively, compared to the corresponding NM plants (Fig. 3b).

The shoot K concentration of NM plants decreased significantly with adding Pb in soil, while K concentration changes in the root were not significant. Inoculation with AM fungus significantly increased the K concentrations of shoots at all levels containing Pb, especially Pb450 level. However, a significant increase in K concentrations of roots due to fungal symbiosis was observed only at Pb150 and Pb300 levels (Table 5).
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Pb stress caused an increase of 39 and 70% in the shoot and root Ca concentrations at the level of Pb150 compared to the control, respectively. Although, a decrease of 11 and 52% (respectively in the shoot and root Ca concentrations) was found at the level of Pb450. Therefore, Pb stress changed the Ca concentration in the root more than in the shoot. Mycorrhizal inoculation significantly increased Ca concentration in the shoots at Pb300 and Pb450 levels and in the roots at Pb150 and Pb450 levels (Table 5).

Mycorrhiza, Pb stress, and their interaction significantly affected Mg and Cu concentrations only in the root (Table 5). Changes in Mg and Cu concentrations under Pb treatments were not significant compared to non-contaminated control, and AM inoculation increased their concentrations only at the Pb150 level significantly compared to corresponding NM plants (Table 5).

The shoot Zn concentration of NM plants at high Pb levels decreased significantly compared to Pb control, and a significant increase in its concentration due to fungal symbiosis was observed at all Pb levels (Table 5). Pb stress caused a significant increase in Zn concentration of NM roots only at the Pb450 level. AM fungus significantly increased the Zn concentration of the root at all Pb levels as the highest concentration was related to AM plants at Pb150 and Pb300 levels, which increased by 137 and 215%, respectively, compared to NM plants (Table 5).

Pb stress caused a significant decrease in shoots and roots Mn concentrations of NM plants. Plant inoculation with AM fungus led to increased Mn concentration in the shoot at Pb300 and Pb450 levels and in the root at all levels containing Pb while decreasing its concentration in the shoots and roots at the Pb control level (Table 5).

All levels containing Pb induced a significant increase in shoots Fe concentration of NM plants, while the root Fe concentration showed a considerable increase in NM plants only at the Pb150 level compared to the control. Symbiosis with *F. mosseae* caused a considerable reduction in shoot and root Fe concentration compared with related NM plants at different Pb levels except for Pb150 level in roots (Table 5).

**Pb Concentration in Plant**

Mycorrhiza, Pb stress, and mycorrhiza×Pb interaction significantly affected shoot and root Pb concentration (Table 2). A significant increase in shoot and root Pb concentrations was observed with increasing Pb in soil, and *F. mosseae* inoculation significantly decreased them at all Pb levels (Fig. 3c,d).

**Phytoremediation Potential and Plant Tolerance**

No significant changes in Pb TF of NM plants were found at different Pb levels except for the Pb300 level compared to control. In NM plants, Pb TF at the Pb450 level was significantly higher than at the Pb300 level. The TF values of AM plants were lower at levels of Pb150, Pb300, and
Table 5 Effect of mycorrhizal inoculation and soil Pb levels on concentrations of mineral nutrients in G. glabra

| Pb stress | AM status | K (mg g⁻¹DW) | Ca (mg g⁻¹DW) | Zn (µg g⁻¹DW) | Fe (µg g⁻¹DW) | Mn (µg g⁻¹DW) | Mg (mg g⁻¹DW) | Cu (µg g⁻¹DW) |
|-----------|-----------|--------------|---------------|----------------|---------------|---------------|---------------|---------------|
|           | Shoot     | Root         | Shoot         | Root           | Shoot         | Root           | Shoot         | Root           |
| Control   | NM        | 18.43 ± 0.641ab | 9.07 ± 0.159bc | 14.06 ± 0.105  | 12.15 ± 0.029d | 20.90 ± 0.687  | 17.80 ± 0.058  | 757.67 ± 55.842d |
|           | AM        | 19.36 ± 0.710a  | 6.68 ± 0.566c  | 12.31 ± 0.299c | 9.97 ± 0.000c  | 34.55 ± 0.491c | 26.98 ± 1.666  | 371.85 ± 80.167 |
| Pb150     | NM        | 15.10 ± 0.553c | 8.81 ± 0.084c  | 15.55 ± 0.179c | 20.70 ± 0.461c | 20.37 ± 1.211c | 22.27 ± 1.224c | 2276.33 ± 140.67 |
|           | AM        | 19.28 ± 0.065a | 11.50 ± 0.226a | 12.35 ± 0.192c | 25.33 ± 0.921c | 335.70 ± 43.476 | 1926.00 ± 81.300ab |
| Pb300     | NM        | 12.22 ± 0.725a | 8.18 ± 0.417c  | 15.04 ± 0.854c | 16.17 ± 1.068c | 166.00 ± 46.146c | 179.83 ± 74.370c | 826.7 ± 3.712 |
|           | AM        | 17.40 ± 0.376c | 9.62 ± 0.166b  | 15.50 ± 0.296c | 28.70 ± 0.173c | 415.50 ± 66.316c | 676.50 ± 85.739c |
| Pb450     | NM        | 6.42 ± 0.599c  | 8.08 ± 0.300c  | 15.53 ± 0.683c | 24.43 ± 1.233c | 1159.00 ± 471.423c | 785.70 ± 101.097c | 1100.5 ± 5.428c |
|           | AM        | 18.21 ± 0.621a | 8.73 ± 0.407c  | 14.05 ± 0.625c | 21.27 ± 1.884c | 422.67 ± 33.174c | 345.15 ± 2.973c | 1165.0 ± 8.038c |

Significance

- Fungi
- Pb stress
- Fungi × Pb stress

Data present the means of four replicates ± standard error. Values labeled with the different letters present significant differences (p < 0.05) according to the Duncan test. Pb150, Pb300, and Pb450 are 150, 300, and 450 mg Pb Kg⁻¹ soil, respectively. NM and AM are non-mycorrhizal and arbuscular mycorrhizal inoculated plants.

Discussion

The results showed the decrease of the shoot growth parameters of liquorice plants under Pb stress, especially at high Pb level (Pb450) (Table 3), which is consistent with the results of other researchers (Dhawi et al. 2016; Yang et al. 2015). Visible symptoms of HM toxicity in plants are stunted growth, chlorosis, root browning, decline, and death (Ozturk et al. 2008). The accumulation of Pb in the soil can inhibit plant growth. The results of R/S in this study showed that Pb stress reduced the shoot growth more than root growth (Table 3). Inhibition of shoot growth at different Pb levels in the soil can inhibit plant growth (Chen et al. 2013). Therefore, the high availability of Pb in the soil can inhibit plant growth (El-Rahman et al. 1974). Therefore, the increase of the R/S ratio could be significant in the Pb tolerance of the liquorice plant. The positive effect of symbiosis with F. mossae on shoot biomass was more efficient than root biomass. It suggests that mycorrhiza is more efficient than non-mycorrhiza in mycorrhiza liquorice due to the role of the hyphal net in transporting nutrients to the roots (Ozkan et al. 2008). The accumulation of Pb in plants is associated with changes in membrane permeability and phytochemistry. The accumulation of Pb in the soil can inhibit plant growth (Hochanadel et al. 2013). Therefore, the high availability of Pb in the soil can inhibit plant growth (El-Rahman et al. 1974). Therefore, the increase of the R/S ratio could be significant in the Pb tolerance of the liquorice plant.

The increased EERSP concentration in Pb soil inoculated with AM fungi significantly reduced the EERSP concentration of control plants inoculated with NM fungi by 15%, 19%, and 39% at different Pb levels. At all different Pb levels, the highest TI of AM and NM plants was observed at the Pb450 level. Also, inoculation with AM fungus significantly increased liquorice TI at levels of Pb150 and Pb300 compared with related NM plants. The BCF values of NM plants in levels containing Pb Pb450 by 23, 19, and 39% than their corresponding AM plants (Fig. 4).
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Fig. 4 Transfer factor (TF) (a), bioaccumulation factor (BCF) (b), and plant tolerance index (TI) (c) of G. glabra non-inoculated (NM) or inoculated with arbuscular mycorrhizal (AM) under different levels of Pb. Pb150, Pb300, and Pb450 are 150, 300, and 450 mg Pb Kg⁻¹ soil, respectively. Error bars indicate standard deviation.

Fig. 5 The Easily glomalin-related soil protein (EE-GRSP) content of G. glabra non-inoculated (NM) or inoculated with arbuscular mycorrhizal (AM) under different levels of Pb. Pb150, Pb300, and Pb450 are 150, 300, and 450 mg Pb Kg⁻¹ soil, respectively. Error bars indicate standard deviation.

Their bioavailability (Raiz et al. 2021). It has been reported that the extensive mycorrhizal hyphae act as the sink of HMs in contaminated soil (Hrishikesh et al. 2010). Therefore, AM fungi improve shoot biomass by retaining HMs in plant root and mycorrhizal hyphae, limiting the translocation of HMs to shoot (Janeeshma and Puthur 2020), as well as sequestering HMs as different chemical forms in subcellular compartments (Raiz et al. 2021).

Photosynthetic Pigments

The present study showed a positive correlation between photosynthetic pigment content and Pb stress so the highest content was observed at the Pb300 level. Consistent with our results, a significant increase in Chl content was observed in leaves of three Atriplex varieties after the plants were exposed to HM stress (Sai Kachout et al. 2015). Atriplex species, as halophytes, are naturally present in environments characterized by an excess of toxic ions (Zhao et al. 2003). It has been reported that the increase of Chl production is considered a biochemical indicator of stress tolerance in different plant species (Jiang et al. 2017). Moreover, carotenoids, as non-enzymatic antioxidants, play an essential role in protecting Chl and membranes from destruction by scavenging reactive oxygen species (ROS) (Yang et al. 2015). It seems that the increase of photosynthetic pigment contents with lead stress in liquorice leads to stress tolerance in this plant.

There are reports of an increase in Chl content as a result of inoculation with AM fungi in some plant species (Zhang et al. 2010; Debeljak et al. 2018). In this study, inoculation with F. mosseae significantly increased the Chl content at levels of control and Pb300 and reduced its content at levels of Pb150 and Pb450. Therefore, the effect of AM fungus on physiological and biochemical parameters and also the tolerance of host plant to HMs depends on the type and concentration of HM (Hildebrandt et al. 2007) and plant genotype (Cui et al. 2019). In this study, the reduction of photosynthetic pigment contents of AM plants was mainly through the dilution effect, which is an increase in the size and biomass of the plant (Cui et al. 2019). Also, Dhawi et al. (2016) reported the upregulation of glutamic acid in Sorghum inoculated with AM fungus. Glutamic acid is a common precursor of proline and Chl biosynthesis in plants. According to the opposite effect of F. mosseae fungus on contents of Chl (Table 4) and proline (Fig. 2a,b) in liquorice at levels of Pb150 and Pb450, proline biosynthesis rather than Chl from glutamic acid seems to be a dominant pathway in AM plants. Proline, as an important osmoprotectant is involved in the maintenance of tissue water content (Hashem et al. 2016). Therefore, proline accumulation in the mycorrhizal liquorice plants improved the growth parameters and diluted the content of Chl in the shoot.

Proline

The more production of proline in plants was considered an adaptive response to HM toxicity (Zhang et al. 2010). Clemens (2006) suggested that water imbalance, which occurs due to HM stress, induces proline biosynthesis. In
metal-stressed plants, the activity of proline is associated with ROS scavenging, which leads to maintaining antioxidant enzyme activities, cellular redox homeostasis, and reconstruction of Chl besides regulation of intracellular pH (Emamverdian et al. 2015). The present study also showed proline accumulation at Pb150 and Pb450 levels, which could be related to more tolerance of liquorice to Pb stress at these levels. However, proline accumulation was not observed at the Pb300 level. It seems that the HM concentration is a crucial factor determining proline accumulation in plant species (Emamverdian et al. 2015).

Proline production induced with AM fungi during HM stress was reported in *Solanum lycopersicum* (Hashem et al. 2016) and *Saccharum spontaneum* (Mirshad and Puthur 2017). Proline accumulation helps plants to maintain cellular water potential well below of the soil solution (Hashem et al. 2016). In this study, more proline production induced with fungus indicates the potential role of *F. mosseae* in maintaining the host plant growth under Pb stress. Also, one of proposed roles of proline is to reduce free radical level. Therefore, *F. mosseae* fungus induces alleviation of ROS under Pb stress in AM plants by strengthening the antioxidative system (Raiz et al. 2021).

**Total Soluble Sugars**

The present study showed an increase in TSS content of the shoot under Pb stress as a tolerance mechanism. In contrast, the amount of root TSS was not affected by P150 and Pb300 levels but significantly decreased at the Pb450 level (Fig. 2c,d). It has been reported that water-soluble carbohydrates play an essential role in plant stress responses (Keunen et al. 2013). Soluble carbohydrates act as the building units and energy providers for plant growth (Muller et al. 2011). They also function as osmoprotectants for stabilizing cellular membranes and maintaining turgor during osmotic stress (Peshev and Van den Ende 2013). Moreover, soluble sugars contribute to ROS scavenging mechanisms. The protective properties of soluble sugar during oxidative stress were generally attributed to direct or indirect signaling triggering the production of ROS scavengers and/or repair enzymes (Keunen et al. 2013). Sugars have the capacity to scavenge OH, where normal enzymatic reduction of this free radical does not exist (Stoyanova et al. 2011).

In general, the results of this study showed the higher TSS content of AM than NM plants (Fig. 2c,d). Similarly, Garg and Aggarwall (2012) reported that symbiosis with *Glomus mosseae* caused an increase in the sugar content of *Cajanus cajan* treated with cadmium stress. It is suggested that AM fungi symbiosis protects the photosystem II reaction center and also improves the gas exchange capacity of stomata in host plants under abiotic stress (Yang et al. 2015). Therefore, by the improvement of photosynthetic parameters, AM plants increase the efficiency of photosynthesis and the production of TSS in comparison with NM plants (Rahimzadeh and Pirzad 2017). In AM plants under stress conditions, TSS can be used to prevent plants from certain deaths and also, maintain and develop of AM fungi in the roots via the supply of necessary energy (Xue et al. 2008; Amanifar and Toghranegar 2020).

**Total Phenolics and Flavonoid**

Most phenolic compounds, as antioxidants, can scavenge the ROS produced during HM stress and cause stress tolerance in plants (Janeeshma and Puthur 2020; Michalak 2006). Phenolics, especially flavonoids, can be oxidized by peroxidase, and contribute to the H$_2$O$_2$-scavenging, phenolic/ascorbate/peroxidase system against HM toxicity (Michalak 2006). Flavonoids may exert their beneficial effects by affecting auxin transport and regulating the root architecture, which could be hindered by abiotic stresses (Potters et al. 2007).

Changes in phenolic compounds have been reported in various plants under HM stress (Hashem et al. 2016; Pazoki 2015). The results of this study showed an increase in root TP content of NM plants at high Pb levels (Pb300 and Pb450) and a decrease in shoot TP content of NM plants at all levels containing Pb (Table 4). The root of many plants exposed to HMs exudes a high level of phenolics (Winkel-Shirley 2002). They may suppress the superoxide-driven Fenton reaction, an important ROS source, probably by chelating iron ions (Michalak 2006). Also, in this study, a significant increase in shoot and root flavonoid content was observed at the Pb450 level compared to the Pb300 level (Table 4). It has been reported that flavonoids protect ROSs by reducing the singlet oxygen, impeding the activity of enzymes engaged in the formation of free radicals, chelating transition metal ions, quenching lipid peroxidation, and recycling other antioxidants (Khalid et al. 2019).

Our results showed mycorrhizal mediated changes of phenolic compounds content in liquorice plants under lead stress. Symbiosis with *F. mosseae* significantly increased the content of shoot TP and flavonoid and reduced the content of root TP and flavonoid at the highest Pb level (Pb450) (Table 4). Higher accumulation of phenolic compounds was reported in mycorrhizal *Solanum lycopersicum* (Hashem et al. 2016) and *Calendula officinalis* (Hristozkova et al. 2016) compared with non-mycorrhizal plants. Higher phenolic compounds in AM plants would provide more antioxidant activity under HMs stress. It has been proposed that metal-induced up-regulation of phenylalanine ammonia-lyases and chalcone synthase genes is associated with mycorrhizal colonization and thereby results in phenolic compounds accumulation in mycorrhizal plants (Zhang et al. 2013). However, a lower accumulation of phenolic
compounds was found in mycorrhized *Medicago truncatula* (Aloui et al. 2012) and *Paxillus-Pinus* (Schützendübel and Polle 2002) in comparison with NM plants. The lower accumulation of phenolic compounds in AM plants may reflect the less oxidative damage by HMs stress (Schützendübel et al. 2002) in comparison with NM plants. The lower accumulation of phenolic compounds in liquorice was dependent on plant organ and lead concentration in soil.

**MDA**

Membrane lipids are considered the primary targets of ROS during HM stress (Fernandez-Fuego et al. 2017). Peroxidation of membrane lipids, which can be indexed by MDA content, results in loss of membrane integrity and function. The increase of lipoxygenase activity during stress initiates the peroxidation of membrane lipids (Djebali et al. 2005). This study also showed the increase of root MDA content at Pb150 and Pb300 levels. Since the roots were in direct contact with Pb stress, oxidative stress was caused by Pb, and consequently, peroxidation of membrane lipids was increased.

In this study, the decrease of MDA content due to *F. mosseae* inoculation confirmed the findings of other researchers (Alqarawi et al. 2014; Fernandez-Fuego et al. 2017; Hashem et al. 2016). AM fungi reduce the production of ROS and MDA by improving phosphorus uptake and inducing an antioxidant defense system, which helps to improve the toxic effects of HMs on host plants (Alqarawi et al. 2014; Janeeshma and Puthur 2020).

**Mineral Nutrition**

In this study, a significant decrease in the concentrations of P, K, Ca, Mn, and Zn in shoots and P, Ca, Mn, and Fe in roots at the Pb450 level was observed. The concentrations of shoot Fe and root Zn increased significantly at the Pb450 level. The physiological changes resulting from the interaction HMs and plant root cells play an essential role in membrane damage and reduced nutrient uptake (Garg et al. 2017). Also, the competition between HMs and mineral nutrients for the same transporters causes different changes in nutrients concentrations (Cui et al. 2019).

Our results showed that the *F. mosseae* inoculation significantly increased the concentrations of P, K, Ca, Zn, and Mn in shoot and roots, and Mg and Cu in roots while it decreased Fe concentration in shoot and root under Pb stress. It was reported that inoculation of AM fungi could increase the concentrations of various nutrients significantly under metal toxicity (Andrade et al. 2010; Cui et al. 2019; Dhawi et al. 2016; Zhang et al. 2015). For instance, mycorrhizal symbiosis increased concentrations of P, K, Ca, Mg, Mn, and Zn in *Sorghum* BTx623 under HMs toxicity (Dhawi et al. 2016). The inoculation of *Coffea arabica* with AM fungus enhanced concentrations of P, K, and Cu in shoots and K, P, Ca, and Mg in roots and decreased those of Ca, Mg, and Mn in shoots under Cu and Zn stress (Andrade et al. 2010). Taken together, these results indicate that mycorrhizal inoculation augments plant Pb tolerance in liquorice by altering the accumulation and partitioning of mineral nutrients in shoots and roots. Also, upregulation of membrane transporters has been reported in AM fungi and in mycorrhizal plants (Ferrol et al. 2016). AM fungi play an essential role in the uptake of inorganic phosphate (Pi) and can supply up to 80% Pi for the host plant (Raiz et al. 2021). The organic acids and the phosphatase secreted by AM fungi can stimulate the solubilization of insoluble phosphate, consequently can improve the absorption and transport of Pi by plants (Raiz et al. 2021). Finally, AM fungi can maintain cellular ionic homeostasis and play a key role in plant resistance under HMs stresses. Since some elements act as cofactors of antioxidant enzymes, ionic balance leads to plant tolerance by improving the activity of these enzymes (Cui et al. 2019). On the other hand, the efficiency of this symbiosis on improving mineral nutrient uptake varies among plants and AM fungi species, type of HM, and extent of metal toxicity (Raiz et al. 2021).

**Accumulation and Translocation of Pb in Plant**

The shoots and roots Pb concentration of liquorice increased significantly with increasing soil Pb concentration however, its concentration in the roots was higher than the shoots (Fig. 3c,d). The reduction in metal translocation rate from roots to shoots is considered a HM tolerance mechanism. It has been reported that the presence of suberin barriers such as Casparian strips in the root endodermis and the lack of specific membrane transporters may lead to more accumulation of Pb in roots (Bin Luo et al. 2014). In this study, the inoculation with *F. mosseae* was effective in the decrease of shoots and roots Pb concentrations (Fig. 3c,d). Since arbuscular mycorrhiza can affect uptake and translocation of HMs in host plants, mycoremediation using these fungi is considered the most effective method among different bioremediation methods. The ability of AM fungi for HMs sequestration inside the fungal structures (Garg et al. 2017), their complexation with hyphal wall compounds such as chitin and chitosan (Galli et al. 2003), and chelating HMs in the rhizosphere by secreted glomalin have been reported (Janeeshma and Puthur 2020).

The results of the present study showed TF values of Pb<1 in NM and AM liquorice plants (Fig. 4a) which indicate the reduced upward movement of Pb and more accumulation of this metal in roots. This observation was in accordance with Tang et al. (2009) and Wu et al. (2016) results. The symbiosis with *F. mosseae* significantly de-
increased TF and BCF values in liquorice at all levels of Pb. Our findings showed that liquorice is a Pb-tolerant species and could be considered a suitable candidate for phytostabilization purposes in Pb contaminated soils. Phytostabilization is an important phytoremediation method that has been reported in AM plants cultivated in Pb contaminated soils (Garg and Aggarwal 2012; Sudová and Vosátka 2007). Phytostabilization is the accumulation of HMs in the plant root system, which decrease their translocation to the shoot system (Radziemska et al. 2017). Also, it reduces the availability and mobility of HMs in the soil and prevents the leaching of HMs and groundwater contamination (Janeeshma and Puthur 2020). Also, our results showed that the inoculation with *F. mosseae* increased TI of the liquorice plant, especially at Pb150 and Pb300 levels (Fig. 4c). AM fungi with improvement in the uptake of mineral nutrients, especially Pi, result in enhanced plant growth and reduced toxicity of HMs due to dilution effect (Garg et al. 2017).

### Colonization Rate and Glomalin

In this study, colonization rate was negatively affected by Pb150 and Pb300 levels while positively affected by Pb450 level (Fig. 1). Reduction and increase of colonization rate by HMs have been reported by other authors (Feng et al. 2013; Hildebrandt et al. 1999; Yang et al. 2008). It has been proposed that host plants may regulate AM colonization, offering them more carbohydrates due to the protective role AM plays against HMs toxicity (Ghasemi Siani et al. 2017). According to the results of this study, it seems that more AM colonization rate of liquorice roots at the high Pb level reduced Pb toxicity. Chen et al. (2005) suggested that the magnitude of Pb inhibition of mycorrhizal colonization differed among plant species. In this study, the inhibition of AM colonization rate occurred at Pb150 and Pb300 levels.

Results showed that the concentration of EE-GRSP, considered a newly synthesized glomalin and relatively more labile, in the AM treatments was higher than NM ones (Fig. 5). AM fungi secrete a water-insoluble and temperature resistance glycoprotein, glomalin which immobilizes HMs in the rhizosphere and decreases their availability for plants (Ghasemi Siani et al. 2017; Wu et al. 2014). Thus, increasing glomalin secretion in AM treatments could indicate the protective mechanism of AM symbiosis to limit Pb content in host plant tissues. Moreover, similar to the results of root colonization rate, the content of EE-GRSP increased as soil Pb concentration increased (Figs. 1 and 5). Both positive and negative correlations between HMs and GRSP concentrations have been reported by Yang et al. (2017). Also, they proposed that AM species, host plant species and development, HM type, and edaphic factors may affect this correlation (Yang et al. 2017).

### Conclusion

The results indicated that liquorice is a Pb-tolerant plant and could be considered a suitable candidate for phytoremediation in Pb contaminated ecosystems. Also, this study showed that AM fungus with improvement in the uptake of mineral nutrients, especially phosphorus, osmotic adjustment (soluble sugars and proline), and antioxidant system (proline, phenolics, and flavonoids) results in enhanced shoot growth and reduced Pb toxicity. Also, AM symbiosis reduced the Pb accumulation in the shoot by immobilizing Pb in the soil with the help of secreted glomalin and decreasing metal translocation rate from root to shoot. The decrease of the Pb accumulation in the shoot can be significant for feeding livestock in environments contaminated with HMs that need further investigation. Generally, this study allowed a deeper insight into the Pb detoxification mechanisms in the liquorice plant and supported the potential use of *F. mosseae* fungus for bioremediation of Pb contaminated soils.

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### Conflict of interest

E. Yeganeh, E. Vatankhah, Z. Toghranegar and S. Amanifar declare that they have no competing interests.

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