Tolerance and Physiological Responses in Two Populations of Harmel Plant to Silver Stress, A Suitable Candidate for Accumulation of Ag

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Abstract

By studying harmel plants in Ag metal-contaminated mineral areas, it was found that harmel plants can accumulate Ag metal, so the present study aimed to investigate the effects of Ag exposure (0, 1, 2.5, 5, 10 mgL^{-1} Ag) to harmel seedlings. Two populations (metallicolous and non-metallicolous) were compared about Ag tolerance, Ag accumulation, translocation factor (TF), photosynthetic pigments, antioxidant enzyme activity and, non-enzyme metabolite. At first, harmel plants were studied for their ability to accumulate silver metal in a silver metal-contaminated mineral area. Also, the results of hydroponic culture showed that the increase of Ag concentrations in the nutrient solution reduced root length, shoot length, root dry weight, shoot dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and, total soluble sugars in both populations, but the accumulation is more pronounced in metallicolous populations than non-metallicolous. In response to this, the antioxidant activities were increased under Ag exposure, and sharp in the metallicolous population. In conclusion, the above results show that harmel seems a suitable candidate for Ag-accumulation; and these findings support the use of harmel as an acceptable species for cultivation in soils that are contaminated with Ag and strategies to minimize the toxicity of Ag in plants.

Introduction

Heavy metals cause contaminants like mining and metal smelting processes, agricultural contaminants including the use of insecticides and municipal wastewater and, municipal contaminants resulting from the use of heavy metal in fuels, paints and, other materials in the soil (Lasat 2000). It is known that soil contamination with nickel, copper, lead and, zinc due to mining activities destroys sensitive plants (Shaw 1989). Plants resist the toxicity of metal ions using two strategies: avoidance and tolerance (Baker 1987). It is evident that different species differ in terms of metal adsorption and, for each species; the metal adsorption is different according to its type.

Few plants can tolerate high concentrations of heavy metals in the soil. Such plants, which accumulate metals in high concentrations in the shoots relative to their roots and without any signs of toxicity, are called hyper-accumulating plants (Baker 1981). Plants containing more than 3000 mg^{-1} kg zinc, 300 mg^{-1} kg copper, cobalt and chromium, 1000 mg^{-1} kg arsenic, nickel and, lead; and for Ag to accumulate 1 mg^{-1} kg dry weight in their aerial parts are considered as hyper-accumulating metal (Van der Ent et al. 2013). In contrast, these concentrations are lethal to ordinary plants (Marschner 1995). Silver nitrate causes severe toxicity in some plant species, bacteria and, algae, so that it has dangerous toxic effects on the flowering, fruiting and physiological mechanisms of plants (Rahmatpour et al. 2017; Tripathi et al. 2017). The highest concentrations of Ag have been reported in bacteria, fungi and, green algae. Little data have been reported on the Ag content of plants. The average content was reported to be 0.25 mg^{-1} kg for algae and 0.06 mg^{-1} kg for plants (Bowen 1966).

The toxicity of heavy metals may be due to the ability of metal ions to bond tightly to oxygen, nitrogen, and sulfur atoms. These atoms are especially abundant in the structure of proteins and generally, the

effect of these metals on protein structures, especially enzymes. In addition, they are increasing the concentration of heavy metals forms free radicals and reactive oxygen species (ROS) (Mahdavian 2021b; Shaw 1989). High concentrations of unnecessary elements lead to symptoms of toxicity in plants (Mahdavian et al. 2016).

Silver is an unnecessary element that can be highly toxic to several plants and animals (Mahdavian et al. 2017; Jacobson et al. 2005). The antimicrobial activity of Ag causes it to complex with membranes, enzymes, nucleic acids and, other cellular compounds (Slawson et al. 1992). Toxicity of Ag has been reported for plant species at a concentration of 75 mg^{-1} kg. Morphological, chloroplasts and, mitochondria damage have been reported in exposed *Potamogeton crispus* L. at concentrations of 5 to 20 µM Ag. Therefore, Ag accumulation in the plant has led to oxidative stress (Ejaz et al. 2018; Xu et al. 2010).

Plants respond to the toxic effects of heavy metal in various ways, such as selective metal uptake, metal attachment to the root surface, metal attachment to the cell wall, and induction of antioxidants. Various antioxidants such as cysteine, non-protein thiol (NP-SH),, ascorbic acid, proline, glutathione and antioxidant enzymes, with which plants may respond to heavy metal. However, the reaction will vary depending on the exposure conditions, metal concentration and, species (Yunxing et al. 2021; Huang et al. 2020; Shahid et al. 2014).

Harmel is a perennial grass-free herb that grows in the Middle East, North Africa, and Central Asia (Shamsa et al. 2007). There are no reports of the effect of Ag on the biochemical and physiological parameters of harmel. Therefore, the present study is by studying harmel plants in Ag metal-contaminated mineral areas; it was found that harmel plants can accumulate Ag metal, then to investigate the toxicity levels of $AgNO_3$ on harmel seedlings in hydroponic conditions. The populations were compared about Ag tolerance, Ag accumulation, translocation factor (TF), photosynthetic pigments, antioxidant enzyme activity and, non-enzyme metabolite.

Materials And Methods

Plant materials and Ag treatments

Harmel seeds were collected from more than forty plants at the mining site of koshk (metallicolous), and non-metallicolous at Kerman, both in Iran. For seed sowing, 9 cm diameter plastic pots containing a mixture of fine and coarse perlite were prepared. Six harmel seeds were planted in each pot and, three replications in each treatment concentration were considered. After five days of irrigation with distilled water, the seedlings were fed for 40 days with a modified nutrient solution of 0.5 Hoagland concentration. After 40 days, the plants were exposed to silver (concentrations of 0, 1, 2.5, 5 and, 10 mgL^{-1} $AgNO_3$) for two weeks. The pH of the nutrient solution and the nutrient solution containing silver were adjusted in the range of 0.5–6.5. The nutrient solutions were replaced with fresh solutions every week and, the plants

were grown in a culture chamber with alternating temperatures of 25/20 ° C (night/day) and light frequency (16 hours of light) (Mahdavian et al. 2016).

Biomass And Ag Concentration Measurement

At the end of the treatment, shoot and root length were measured using a ruler based on centimeters. Also, to measure dry weight, the samples were dried and weighed at 70 ° C.

In order to determine and measure the amount of accumulated metal in the shoot and root portion of the plant between 0.05 and 0.1 gram of plants, dried, crushed and, poured into glass tubes. Then, 4 ml of 37% hydrochloric acid, 4 ml of nitric acid 65%, 1.5 ml of perchloric acid, 1.5 ml of hydrogen peroxide were added to each sample, then samples in the sand bath 150–200°C for 2 Clock was placed. Ag Was measured using a flame atomic absorption spectrophotometer, as described in Reeves et al. (1999).

After collecting soil from 6 sites in the silver-contaminated mineral area, the amount of total and exchangeable silver element (mg^{-1} kg dry weight) of the collected soils was measured according to Faucon et al. (2007).

Translocation Factor (Tf) Measurement

The silver uptake, translocation, and accumulation in harmel were determined by calculating the translocation factor. The translocation factor indicates the ability of plants to translocate heavy metals from roots to shoots and is calculated by dividing the metal concentrations in shoots with that in the roots (Mattina et al. 2003).

Photosynthetic Pigments

Fresh leaf tissues were ground with a mortar and pestle under liquid N_2. Eighty percent (v/v) acetone was added to extract pigments and, after centrifugation of the supernatant for 10 min at 10,000 rpm, OD was measured at 470, 646.8 and, 663.2 nm using a spectrophotometer. The extinction coefficients and the equations reported by Lichtenthaler (1987) were used to calculate the amounts of chlorophyll *a*, *b and* carotenoids.

Assay Of Non-enzymatic Physiological

The phenol-sulfuric acid method was used to measure soluble carbon hydrates (Dubois et al. 1956).

Concentrations of anthocyanin, ascorbate (ASC), dehydroascorbate (DHA) and, reduced glutathione (GSH) were estimated by Wagner (1979), De Pinto et al. (1999) and, Ellman (1959).

To measure the amount of reduced glutathione, 0.5 g of fresh leaf tissue in 4 ml of 15% metaphosphate was ground and, the extract was centrifuged at 10,000 g for 30 minutes at 4 ° C. To 200 µl of the centrifuged supernatant, 2.6 ml of sodium phosphate buffer (pH = 7.7) and 200 µl of 5, 5- Dithio- bis (2-Nitrobenzoic acid) (DTNB) solution (39.6 mg of DTNB dissolved in 20 ml of sodium phosphate buffer) were added. The 30-minute absorbance of the samples was read at 412 nm (Ellman 1959).

Enzyme Extraction And Assays

Fresh leaf samples (1.0 g) were homogenized in 6 ml of cold 50 mM potassium phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP) in an ice bath, was using mortar and pestle. The homogenate was centrifuged for 20 min at 12,000 rpm at four ^{0}C, and the supernatant was used to measure enzyme activities.

Superoxide dismutase, lipooxygenase, guaiacol peroxidase and, catalase activity were determined based on Giannopolitis and Ries (1977), Doderer et al. (1992), Plewa et al. (1991) and, Aebi (1983). Also, ascorbate peroxidase activity was measured according to Nakano and Asada (1981) and Boominathan and Doran (2002).

Statistical analysis

The data were presented by two-way ANOVA, with Ag exposure concentration and population as fixed factors, and individual means were compared using Tukey’s test with $P < 0.05$ as a significance threshold.

Results

The amount of silver in the soil and plants of the mining site

The amount of total and exchangeable silver element (mg^{-1} kg dry weight) of the collected soils is shown in Table 1. According to the results, the total amount of silver in this region is 0.3 to 6.5 mg^{-1} kg dry weight. Also, as shown in Table 1, the exchangeable amount of silver in the pavilion soil is less than 0.1 to 0.5 mg^{-1} kg dry weight. The mean pH of soil samples ranged from 6.8 to 8.8 (Table 1). The amount of silver in harmel is in the range of 0.1 to 0.6 mg^{-1} kg in the roots and 0.2 to 0.3 mg^{-1} kg in the shoots and TF 0.5 to 2.0 (Table 2).

Table 1

Characteristics of soil samples collected (average and minimum-maximum) from silver metal contaminated mineral area

Site	number of samples	Soil pH	Soil EC	Total silver	Exchangeable silver
			(ms cm^{-1})	(mg kg^{-1})	(mg kg^{-1})
1	10	7.5 ± 0.5 7.1–8.3	2.3 ± 1.1 1.1–3.4	1.3 ± 1.1 0.3–2.9	< 0.1
2	10	7.0 ± 0.1 7.0-7.1	8.2 ± 0.1 8.1–8.3	1.6 ± 0.5 0.3–1.7	< 0.1
3	5	6.8 ± 0.3 6.8–7.1	8.6 ± 0.4 8.2-9.0	3.4 ± 1.1 1.6–4.3	0.3 ± 0.1 0.2–0.4
4	5	7.1 ± 0.3 6.9–7.5	8.7 ± 0.5 8.2–9.2	1.4 ± 0.3 0.3–1.5	0.1 ± 0.1 < 0.3
5	6	7.7 ± 0.1 7.7–7.8	3.9 ± 0.1 3.8-4.0	4.5 ± 2.4 0.6–6.3	0.3 ± 0.1 < 0.5
6	10	8.8 ± 0.2 8.6–8.8	1.1 ± 0.1 1.1–1.2	5.8 ± 1.6 1.0-6.5	0.4 ± 0.2 0.1–0.5

Mean and range of silver concentrations (mg kg^{-1} dry weight) in soils, plants (shoots and roots) and TF collected from silver metal contaminated mineral area

Table 2

Mean and range of silver concentrations (mg kg^{-1} dry weight) in soils, plants (shoots and roots) and TF collected from silver metal contaminated mineral area

	silver			
	Root	Shoot	Soil	TF
	0.1-0.6	0.2-0.3	1.0-3.7	0.5-2.0
Peganum harmala L.	0.3±0.1	0.2±0.1	2.3±0.9	1.2±0.5

Plant Growth

According to Fig. 1, there is a significant difference in shoot dry weight between different concentrations of Ag in each population. So that the lowest value at a concentration of 10 mgL^{-1} Ag in both metallicolous populations and non-metallicolous is 67.2 and 66.8% dry weight loss compared to the control, respectively. Also, there is no significant difference between the two populations. Also, the results of the analysis of variance showed that the interaction between population and Ag treatment showed a significant effect on shoot dry weight ($P < 0.05$). Also, under doses of 5 and 10 mgL^{-1} Ag, dry root weight in both harmel populations showed a significant decrease compared to the control. There is no significant difference between the two populations in different concentrations. Also, the results of the analysis of variance showed that the interaction between population and Ag treatment did not have a significant effect on root dry weight (Fig. 1a,b).

Figure 1 (c,d), shows that Ag treatment significantly reduced the length of the shoot and root compared to the control in both populations. In terms of shoot length, there was a significant difference in the levels of 5% in different concentrations in each population, so that at 10 mgL^{-1} concentration in both populations, the lowest shoot length was observed compared to control. Also, the results of ANOVA showed that interaction between population and treatment had no significant effect on root and shoot length.

Ag Accumulation

Based on Fig. 2, Ag treatment at all concentrations caused a significant increase in Ag concentration in shoots and roots compared to control plants in both populations. The results showed a significant difference between the two populations at concentrations of 1, 2.5, 5 and, 10 mgL^{-1}, so that the highest concentration of shoot and root Ag at a concentration of 10 mgL^{-1} of in metallicolous populations than non-metallicolous was observed. Also, analysis of variance showed that the interaction effect of population and Ag treatment on shoot and root Ag concentration was significant ($P < 0.01$).

Analysis of variance showed that the interaction effect of population and silver treatment on TF in harmel plants. The translocation factor of harmel increased as silver concentration enhanced in the cultivation (Fig. 2). The maximum increase in translocation factor value was recorded under silver 10 mgL^{-1}; as compared to the control plants, the transfer factor is less than one.

Photosynthetic Pigments

Based on Fig. 3, there is a significant difference in a, b and, total chlorophyll content between different concentrations of Ag in each population. The lowest total chlorophyll was observed in concentrations of 2.5, 5 and, 10 mgL^{-1} Ag in both populations. Also, there is no significant difference between the two populations in different concentrations. According to the results of the analysis of variance, the interaction between population and Ag treatment did not show a significant effect on chlorophyll content.

Based on Fig. 3d, Ag treatment at all concentrations significantly reduced carotenoid content in both populations of harmel compared to the control. There is no significant difference between the two populations. Also, the results of the analysis of variance showed that the interaction of population and Ag treatment had no significant effect on carotenoid content.

Anthocyanin And Soluble Sugars Concentrations

According to Fig. 4a, it is observed that under the values of 5 and 10 $mg^{-1}L$ Ag, the amount of anthocyanin in both populations of harmel showed a significant increase compared to the control. There is no significant difference between the two populations. The results also showed that the interaction between population and Ag treatment had no significant effect on number anthocyanins.

According to Fig. 4b, it is clear that Ag treatment at all concentrations caused a significant reduction in soluble sugars in both populations of harmel compared to the control. The results showed a significant difference between the two populations at concentrations of 1, 2.5, 5 and, 10 $mg^{-1}L$, so that the lowest amount of soluble sugars in the treatment of 10 $mg^{-1}L$ non-metallicolous population is observed, which reduces 62% compared to the control. Analysis of variance showed that the interaction between population and Ag treatment on the concentration of soluble sugars was significant ($P < 0.05$).

Ascorbate (Asc), Dehydroascorbate (Dha) And, Glutathione (Gsh) Concentrations

Based on Fig. 5a, in different concentrations of Ag treatment, ASC content showed a significant increase compared to the control in both populations. The lowest amount of ASC in control plants and the highest ASC in Ag treatment of 5 mgL^{-1} in metallicolous and non-metallicolous populations were observed with 306.9 and 351.3% increase compared to the control, respectively. The results of the analysis of variance showed that the interaction between population and Ag treatment had a significant effect on ASC content ($P < 0.01$).

Based on Fig. 5b, at different concentrations of Ag treatment, the content of DHA content showed a significant increase compared to the control in both populations. The results showed a significant difference between the two populations, so that the highest amount of DHA was observed in the treatment of Ag 10 mgL^{-1} of the metallicolous population, which increased by 188% compared to the control. Also, the analysis of variance showed that the interaction between population and Ag treatment on the amount of DHA was significant ($P < 0.05$).

Reduced glutathione content is one of the most critical antioxidant indicators of plants in the face of stresses such as heavy metals. Based on Fig. 5c, the amount of GSH in different concentrations of Ag treatment showed a significant increase compared to the control in both populations. The highest GSH in Ag treatment of 10 mgL^{-1} in both metallicolous populations and non-metallicolous increased by 40.7%

and 46.1%, respectively, compared to the control. The analysis of variance showed that the interaction between population and Ag treatment had a significant effect on the amount of GSH ($P < 0.01$).

Antioxidant Enzyme Activities

Based on Fig. 6a, at different concentrations of Ag treatment, catalase (CAT) activity showed a significant increase compared to the control in both populations. The results showed a significant difference between the two populations at concentrations of 5 and 10 mgL^{-1}, so that the highest CAT activity was observed in the treatment of Ag 10 $mg^{-1}L$ metallicolous population, which was an increase of 595.7% compared to the control plant. Also, the analysis of variance showed that the interaction of population and Ag treatment on CAT activity was significant ($P < 0.01$).

It is observed that under the values of 2.5, 5 and, 10 $mg^{-1}L$ Ag, the activity of guaiacol peroxidase (GPX) in both populations of harmel showed a significant increase compared to the control (Fig. 6b). Also, no significant difference was observed between the two populations and, the analysis of variance showed that the interaction between the population and Ag treatment did not show a significant effect on GPX activity ($P > 0.05$).

According to Fig. 6c; it is observed that under different concentrations of Ag, ascorbate peroxidase (APX) activity in both populations of harmel showed a significant increase compared to the control. The lowest APX activity was observed in the control plant and the highest APX activity was observed in 5 and 10 mgL^{-1} Ag treatment in both populations. Also, no significant difference was observed between the two populations and, the analysis of the variance table showed that the interaction between the population and Ag treatment did not show a significant effect on APX activity ($P > 0.05$).

Also, concentrations of 5 and 10 mgL^{-1} Ag caused a significant increase in lipoxygenase (LOX) activity compared to the control in both populations (Fig. 6d). The highest LOX activity was observed in the treatment of 10 mgL^{-1} Ag in both populations. Also, there is no significant difference between the two populations. The results of the analysis of variance showed that population interaction was not significant in Ag treatment ($P > 0.05$).

Superoxide dismutase (SOD) activity showed a significant difference between the two populations at concentrations of 1, 2.5, 5 and, 10 mgL^{-1} (Fig. 6e). The highest superoxide dismutase activity was observed in the treatment of Ag 10 mgL^{-1} in the metallicolous population, which was almost 80.7% higher than the control plant. Also, the analysis of variance showed that the interaction effect of population and Ag treatment on superoxide dismutase activity was significant ($P < 0.01$).

Discussion

In this study, the amount of total Ag in harmel in contaminated soils ranged from 0.1 to 3.7 mg^{-1} kg, with an average of 2.3 mg^{-1} kg. Heavy metals are not insoluble in plants, and are therefore not directly toxic. However, soluble and exchangeable forms may be directly available to soil-based organisms (Lorenz et al. 1997; Pollard et al. 2002; Mahdavian et al. 2017). According to this study, the results showed that the highest exchangeable concentrations of Ag were 0.5 mg^{-1} kg. Although these values suggested a significant increase in availability, the toxicity of an element to a particular organism could not be easily estimated by the concentration of the element insoluble and exchangeable forms alone (Otero et al. 2012). In addition to the concentration of elements in the soil, soil conditions are also fundamental in the uptake of metals by plants. The results of the present study showed that the pH of soil samples is neutral to alkaline. In this pH range, the availability of most heavy metals is low compared to acidic soils (Wong 2003; Harris et al. 1996). Because the concentration of heavy Ag metal in soil samples was high, but the ability to transfer these metals to the root and shoot parts of plants was not good. According to various studies, the availability of most heavy metals in soils with low acidity is higher. Therefore, low transfer can be due to the neutral pH to the play of soils in the mineral zone. Metal concentrations in plants vary between plant species (Quezada-Hinojosa et al. 2015; Alloway et al. 1990). Kabata-Pendias (2011) reported that 0.03 to 0.5 mg^{-1} kg Ag is present in plants grown on unpolluted soils. Ag is one of the most toxic metals and, its concentration in plant tissues is usually less than 0.01 mg^{-1} kg, although it can be higher in plants than the Ag mining areas. Also, in this study, the amount of Ag in harmel is in the range of 0.1 to 0.6 mg^{-1} kg in the roots and 0.2 to 0.3 mg^{-1} kg in the shoots. According to the results of the mineral area obtained from this research, the harmel plant can accumulate silver metal in roots and shoots. The translocation factor (TF) calculates the potency of plants to transfer metals from the root to the shoot. It is given by the ratio of metal concentration in shoot and root. In this research, the TF of harmel enhanced as Ag concentration increased in the soil. Plants with TF higher than one will transfer heavy metals to shoot (Adesodun et al. 2010). While, plants with TF lower than one show lower capability transfer of heavy metal from root to shoot (Mahdavian 2021a; Aran et al. 2017).

In the present study, by examining the effect of $AgNo_3$ treatment on harmel plants, it was found that $AgNo_3$ reduced the growth parameters in both populations, which is due to the decrease in growth parameters, increased Ag uptake by plants (Khan et al. 2019). The results present study showed that the reduction in root and shoot length was observed in the treatment with different concentrations of Ag ions, which is significant at the level of 5%. Similar results have been reported in barley (Fayez et al. 2017) and *Pennisetum glaucum* (Khan et al. 2019). High concentrations of $AgNo_3$ increase ethylene production. Thus, ethylene disrupts auxin transport and reduces growth (Lentini et al. 1988). Jiang et al. (2012) reported that the dry weight of *Spirodela polyrhiza* decreased under the influence of Ag treatment.

Based on the results obtained from the effect of different concentrations of Ag under hydroponic conditions, it was found that in both populations studied in this study, the amount of Ag in the roots increases with the increasing concentration of Ag treatment in the nutrient solution. Also, the concentration of Ag in the root is higher than the shoot part. The concentration of Ag in the shoots and roots of the non-metallicolous population was significantly lower than the metallicolous population in the

Ag treatment, which indicates the more extraordinary ability of the metallicolous population to transfer Ag from the roots to the shoots. It has also been reported that Ag metal tends to accumulate more in roots than in leaves (Smith and Carson 1977). Jiang et al. (2012) reported that Ag accumulation increased in plant tissues of *Spirodela polyrhiza*. An increase in Ag concentration in shoots of *Ocimum basilicum* L. under Ag stress was also reported (Nejatzadeh-Barandozi et al. 2014).

Silver has been shown to interfere with chlorophyll biosynthesis (Davies et al. 1990). Reduction of iron and magnesium with Ag is a reason to reduce chlorophyll formation. Changes in chloroplast structure due to high Ag content are a reason for the decrease in chlorophyll content (Xu et al. 2003; Hu et al. 2007). Chlorophyll a and carotenoids have also been found to be more sensitive to Ag stress than chlorophyll b. The reduction of these pigments has a direct reduction in photosynthetic activity, and therefore reduced carbon stabilization (Baker and Walker 1990). High concentrations of silver nitrate increase ethylene production. Thus ethylene reduces the chlorophyll content (Lentini et al. 1988). Also, Xu et al. (2010) reported that silver nitrate reduced the activity of photosynthetic pigments in *Potamogeton crispus* L. and Khan et al. (2019) in *Pennisetum glaucum* L.

One of the phenolic compounds is anthocyanin, which increases in response to various oxidative stresses (Doong et al. 1993). Also, the role of anthocyanins in the detoxification of heavy metals through the formation of metal-anthocyanin complexes (Boulton 2001). To counteract the oxidative stress induced by heavy metals in plants, there are several high-performance antioxidant compounds that can scavenge free radicals. Phenolic compounds, including anthocyanins, are the most important antioxidant compounds in the plant. These compounds not only kill active free radicals, but also prevent their further production in the stressed plant (Mahdavian 2021b; Winkel-Shirley 2002). The role of anthocyanins in the suppression of free radicals is well established. Heavy metal phenolic compounds protect plant cells from oxidative damage due to stress caused by indirect effects on the collection, destruction, and inactivation of free radicals (Foyer et al. 1997). Also, Abbasi and Jamei (2019) reported that silver nitrate incresed the activity of anthocyanins in *Echium amoenum*.

The increase of soluble sugars in most stressful conditions is a mechanism of stress tolerance and fact regulates the cell water potential in the cytosol to cope with the high concentration of adsorbed ions and accumulated in the vacuole. By reducing the transfer of water to the leaves and following the accumulation of cadmium in the cells, the number of soluble sugars in the plant increases. This feature is a method of plant adaptation to maintain osmotic conditions. In addition, increasing soluble sugars help the plant maintain its carbohydrate stores to maintain optimal basal metabolism under stress. Verma and Dubey (2001) reported that cadmium-induced stress increased soluble sugars in two varieties of rice. The results of the present study also showed that the number of soluble sugars in the harmel plant in both populations increased under the influence of Ag treatment.

Plants use a variety of defense mechanisms to control and neutralize oxygen-induced free radicals. They have an antioxidant defense system with enzymatic and non-enzymatic mechanisms that can scavenge oxygen-free radicals and mitigate the damage caused by oxidative stress (Pandey et al. 2002). The non-

enzymatic defense system in plants induces the synthesis of some antioxidant compounds such as anthocyanins, carotenoids, and ascorbic acid. These antioxidant compounds react with free radicals and, by giving electrons to these reactive radicals, convert them into their stable form (Zhang et al. 2021; Tripathi et al. 2006). Ascorbic acid is a water-soluble compound with high antioxidant capacity in plant cells. This compound is a potent reductant for reactive oxygen species that can kill free radicals directly or enzyme-mediated. The indirect role of ascorbic acid as an antioxidant compound is tocopherol reduction. Tocopherol is a potent antioxidant attached to plant cell membranes that scavenge peroxide and oxygen radicals resulting from oxidative stress (Lea and Leegood 1999; Buchanan et al. 2004). The activity of the ascorbate-glutathione cycle plays a vital role in the suppression of reactive oxygen species and the plant's resistance to oxidative stress. In plants that are exposed to oxidative stress, the destruction of hydrogen peroxide radicals is considered to be the most essential activity of the enzyme ascorbate peroxidase. This enzyme uses the ascorbate substrate as an electron donor (Panda and Choudhary 2005). The results of the present study are consistent with the findings of Smeets et al. (2005) and Cuypers et al. (2001).

Toxicity of Ag treatment causes the production of ROS and oxidative stress. Plants have antioxidant defense mechanisms to deal with these oxidative stress conditions (Jiang et al. 2017; Mahdavian et al. 2016; Huang et al. 2019). Studies mentioned above, showed a significant increase in the activity of the defense system of antioxidant enzymes under Ag treatment compared to control plants. As shown in the results, in plants treated with lead, zinc, and silver, the activity of superoxide dismutase and catalase enzymes was significantly increased compared to the control plant. The enzyme superoxide dismutase plays an essential role in the radical conversion of superoxide to hydrogen peroxide, and catalase is one of the major enzymes in the breakdown of hydrogen peroxide (Del-Rio et al. 1983; Khatun et al. 2008). Increased activity of these enzymes indicates the production of ROS due to the uptake of these metals (Bai et al. 2021; Apel and Hirt 2004; Fabre et al. 2000). Xu et al. (2010) reported that the activity of SOD and CAT in the leaves of *Potamogeton crispus* L. under Ag treatment was due to the increase in the production of free radicals.

Conclusion

In this study, among the studied two populations, plants grown from seeds collected from metallicolous, in higher concentrations of Ag showed a higher tolerance and physiological responses, which indicates their higher compatibility to soils contaminated with Ag. Our results show that $AgNO_3$ further reduces photosynthetic pigments and total soluble sugars, leading to more significant yield loss due to high toxicity. From growth responses and antioxidant enzyme activity, it can conclude that the metallicolous population of harmel demonstrated higher tolerance to Ag than the non-metallicolous population. Both populations were evenly tolerant of Ag, but the metallicolous plants exhibited high levels of Ag accumulation in both roots and shoots. This finding supports the use of *P. harmala* as a suitable plant for cultivation in soils contaminated with Ag and strategies to minimize the toxicity of Ag in plants.

Declarations

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval

This article is original and not published elsewhere. All authors discussed the results, read and approved the final manuscript. The authors confirm that there are no ethical issues in the publication of the manuscript.

Data Availability

All data generated or analyzed during this study are included in this article.

Declaration statements The authors declare that they have no conflict of interest.

Author Contribution

K. M contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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Figures

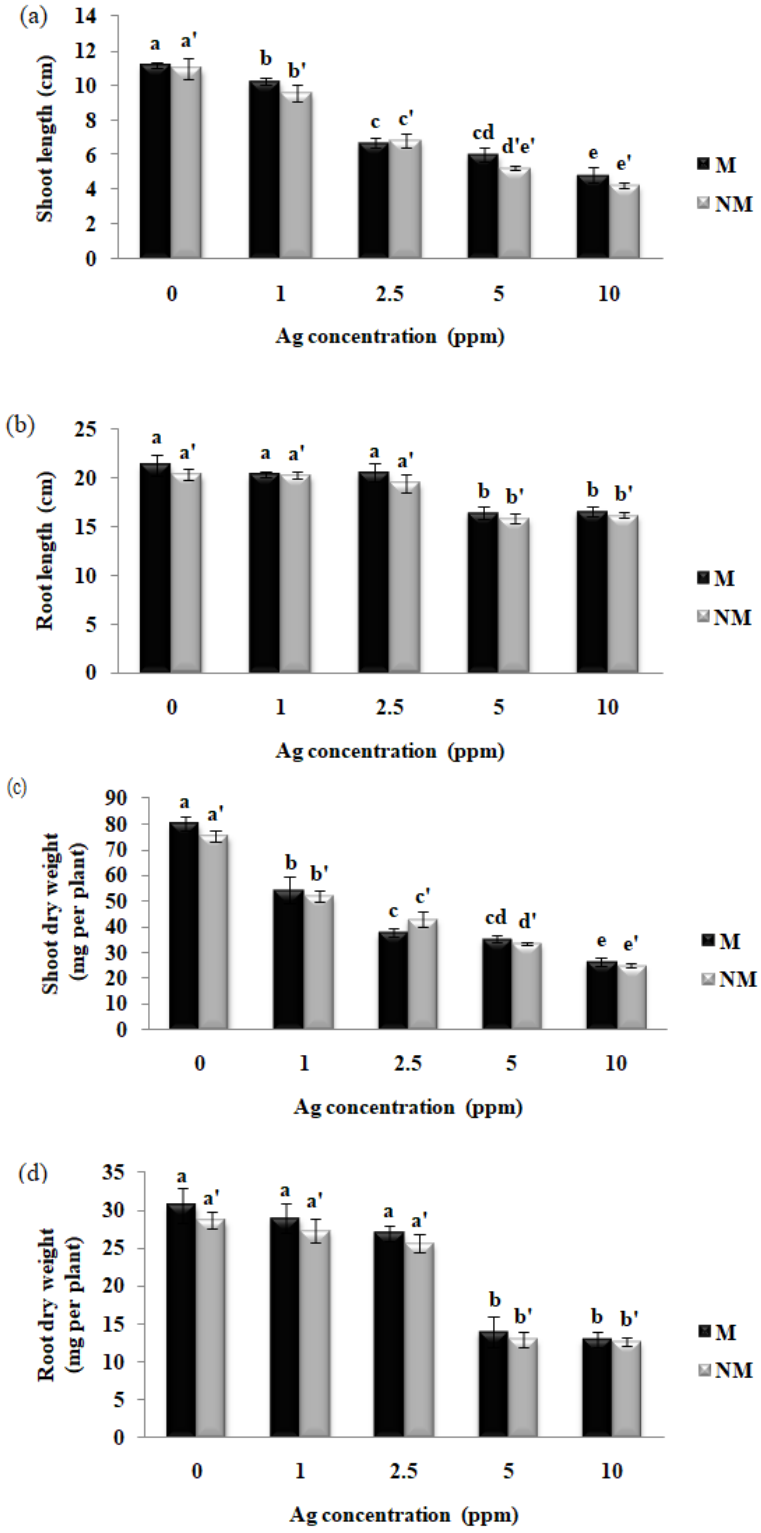

Figure 1

Effect of Ag on the shoot (a) and root (b) length, shoot (c) and root (d) dry weight in two populations of harmel (mean ± SE, n = 12) after exposure to increasing Ag concentrations (mgL-1) for two weeks. Different letters indicate significant differences ($P < 0.05$) among the treatments and control.

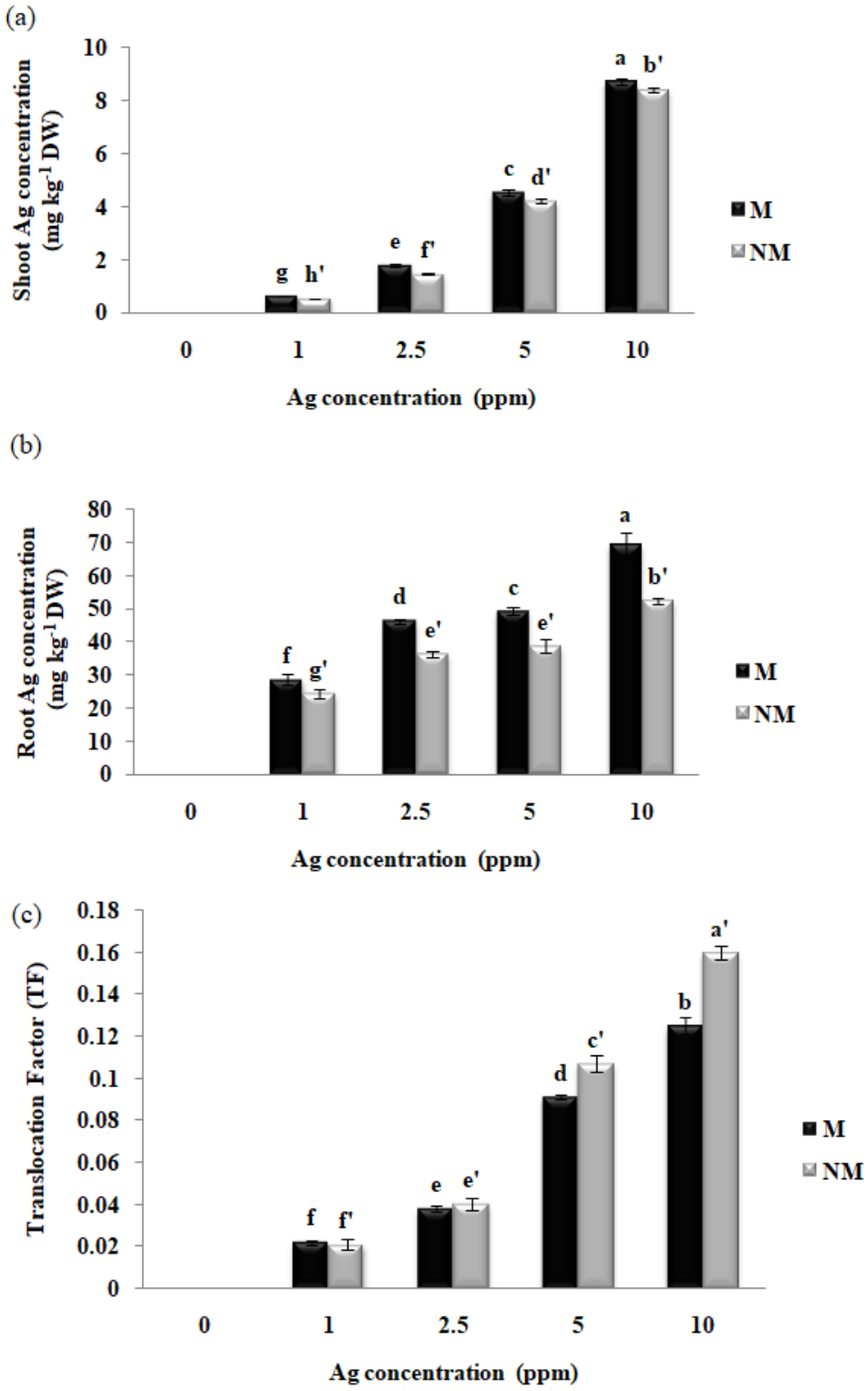

Figure 2

Effect of Ag on the shoot (a) and root (b) Ag concentration and TF (c) in two populations of harmel (mean ± SE, n = 12) after exposure to increasing Ag concentrations (mgL-1) for two weeks. Different letters indicate significant differences ($P < 0.05$) among the treatments and control.

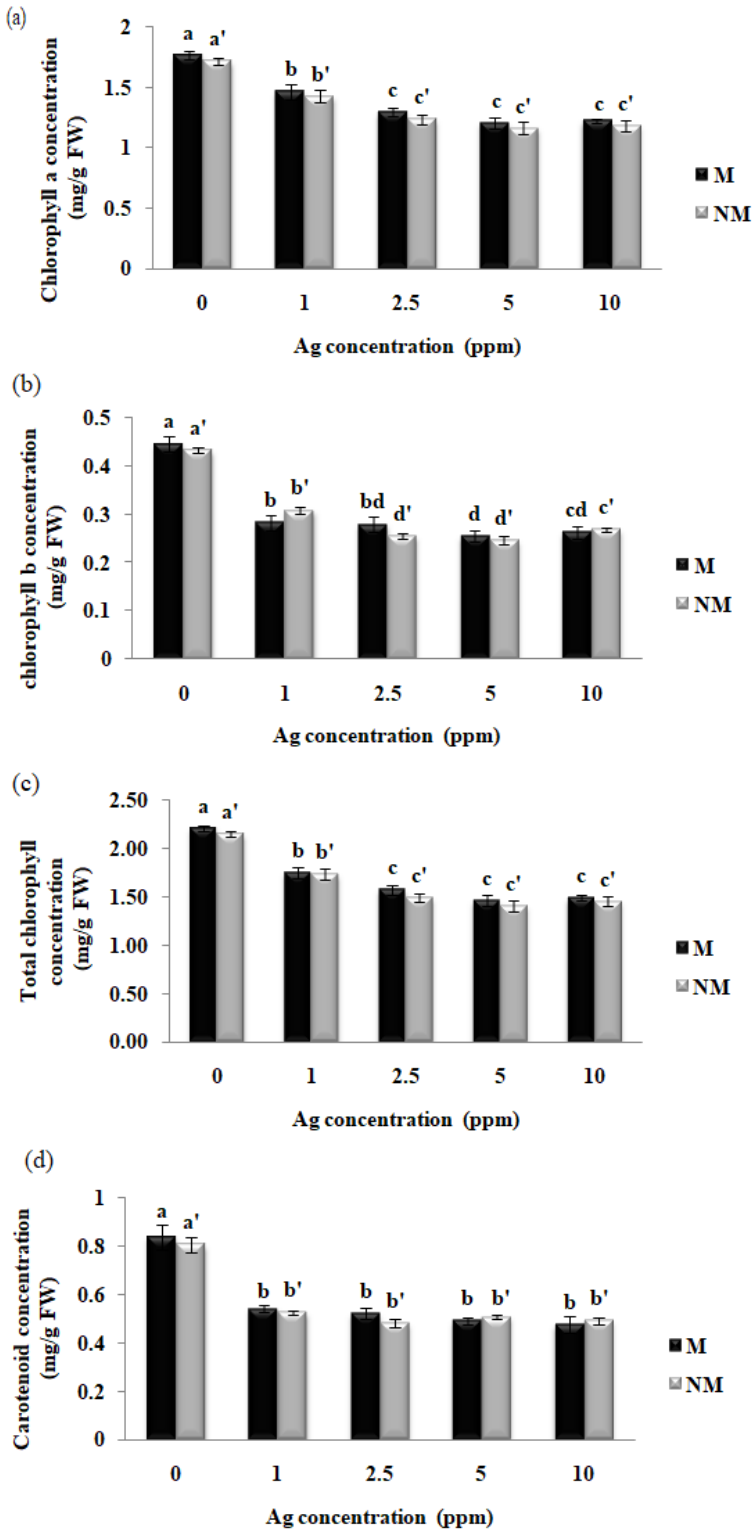

Figure 3

Effect of Ag on on Chla (a), Chlb (b), total Chl (c), and carotenoid (d) contents in two populations of harmel (mean ± SE, n = 12) after exposure to increasing Ag concentrations (mgL-1) for two weeks. Different letters indicate significant differences ($P < 0.05$) among the treatments and control.

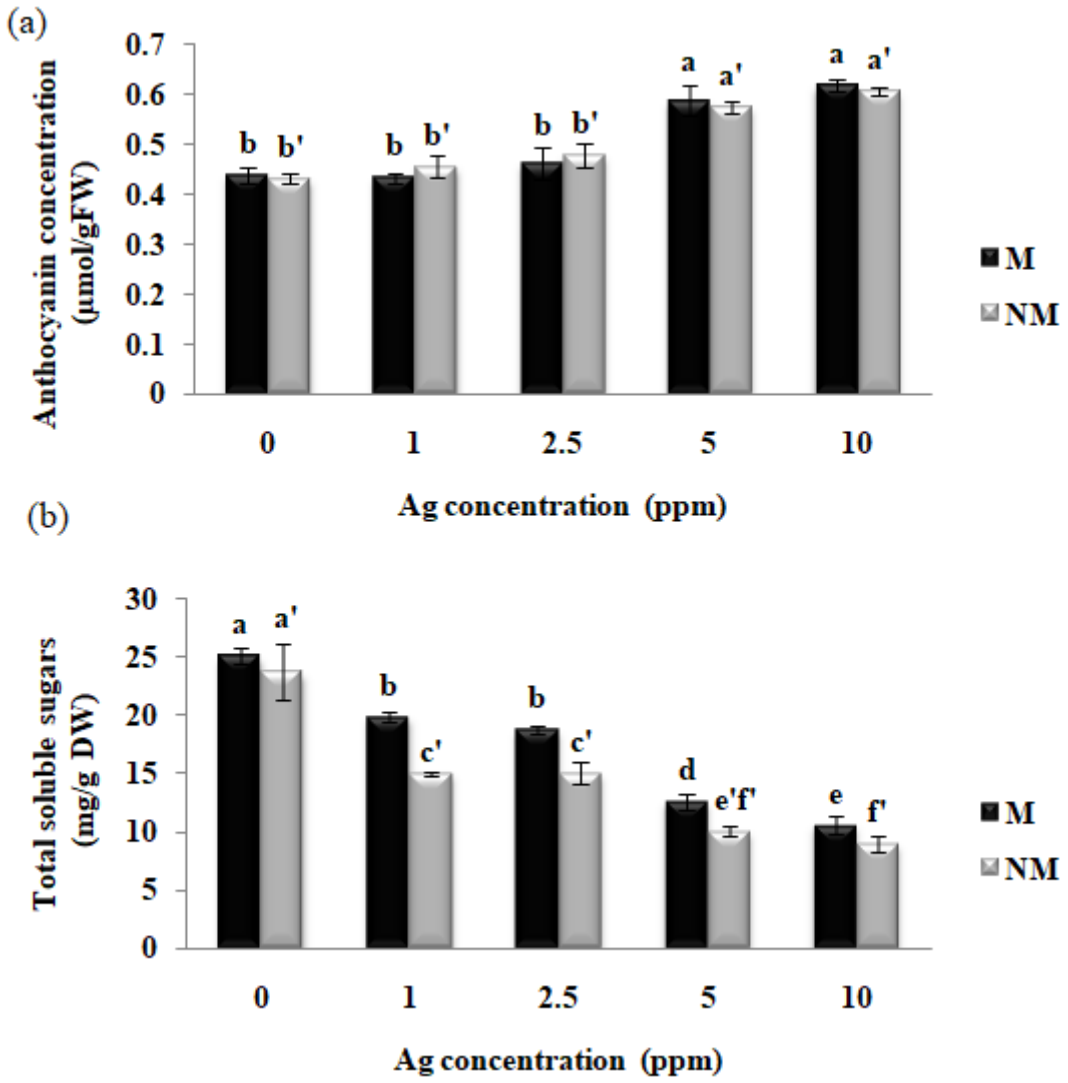

Figure 4

Effect of Ag on anthocyanin (a) and total soluble sugars (b) concentration in two populations of harmel (mean ± SE, n = 12) after exposure to increasing Ag concentrations (mgL-1) for two weeks. Different letters indicate significant differences ($P < 0.05$) among the treatments and control.

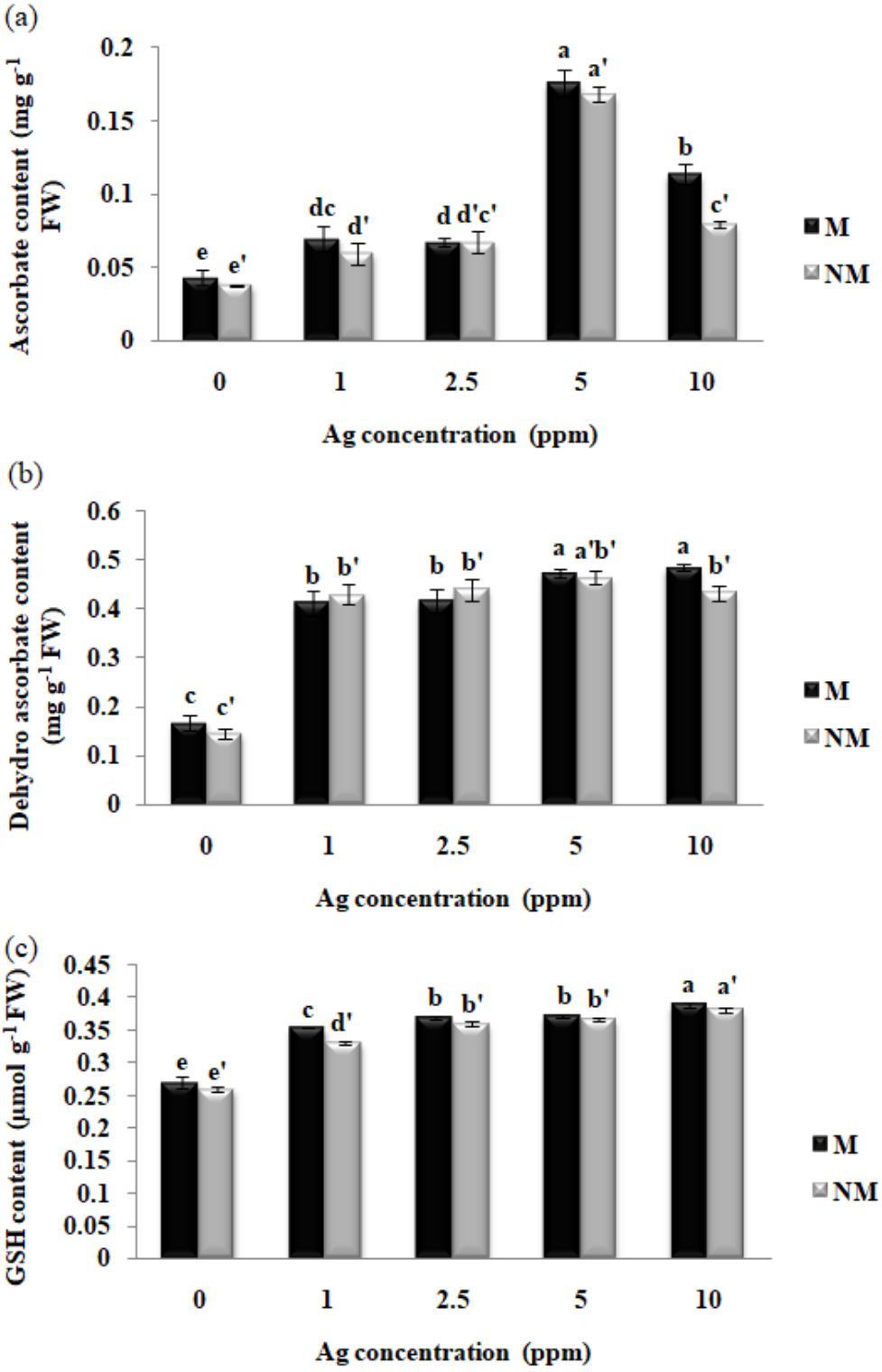

Figure 5

Effect of Ag on ascorbate (ASC) (a), dehydroascorbate (DHA) (b) and glutathione (GSH) (c) concentrations in two populations of harmel (mean ± SE, n = 12) after exposure to increasing Ag concentrations (mgL-1) for two weeks. Different letters indicate significant differences ($P < 0.05$) among the treatments and control.

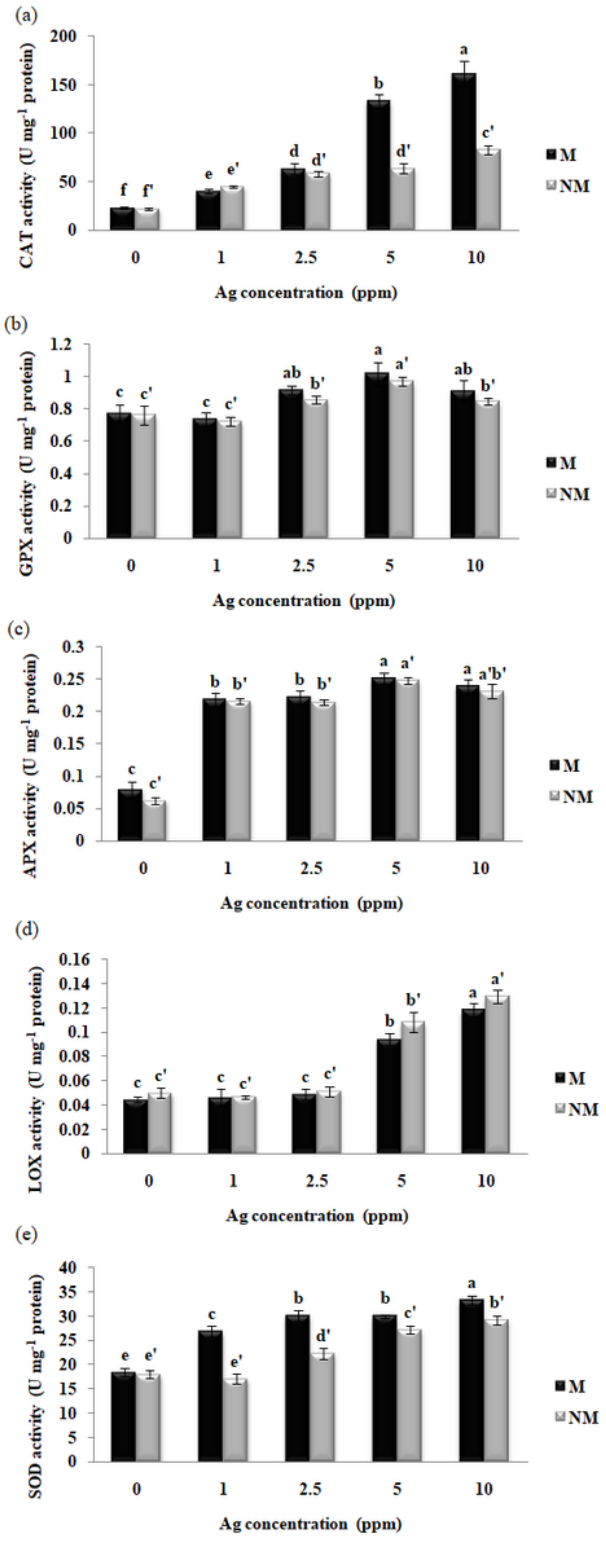

Figure 6

Effect of Ag on the enzyme activities in two populations of harmel (mean ± SE, n = 12) after exposure to increasing Ag concentrations (mgL-1) for two weeks. Different letters indicate significant differences ($P < 0.05$) among the treatments and control.