Differential responses of growth, metals accumulation, oxidative stress and Osmo-compatible solutes in the Halophyte Tamarix gallica under Arsenic and Aluminum stress alone or combined with salt

Dhouha Belhadj SGHAIER (dhouhasghaier@hotmail.fr)
UR MaNE, Université de Carthage
Bernardo Duarte
University of Lisbon
Isabel CAÇADOR
University of Lisbon
Noomene SLEIMI
UR MaNE, Université de Carthage

Research Article

Keywords: Al, As, salt addition, combined stress, enzymes activities, osmotic solutes.

Posted Date: July 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1839955/v1

Abstract

In order to evaluate the ecological risk generated by the heavy metals cumulation in salt-affected soils. The research was carried out in the halophyte *Tamarix gallica* which is known to thrive under harsh environmental occurrences. The main aim of this work was to study the tolerance and accumulation capabilities of *Tamarix gallica* under Arsenic, Aluminium and salt stress. Plants were cultivated in the presence of increasing concentrations of Al and As alone (200, 500 and 800 µM), or mixed with NaCl (200 mM). The results revealed that both metals altered the growth and development of *T.gallica*. Finding showed that the metals' accumulation was dependent on the concentrations of the metals in the medium. Interestingly, while salt reduced As uptake, an augmentation in Al accumulation was observed. In addition, *T. gallica* demonstrated that APX was the predominant enzyme to scavenge excess ROS. However, SOD and GPX were sensitive to the metal, showing different behaviour in metal stress alone or combined with salt. Proline accumulated in a dose-dependent manner under Al and As stress, rather than lower or unchanged levels of glycine betaine. We also noticed a diminution in proline content caused by NaCl application while an increase in glycine betaine was remarked. On the other hand, the combined stress enhanced proline whereas unchanged levels of glycine betaine were observed. Finally, *T. gallica* demonstrated its potential to grow under elevated concentrations of As/Al and could be considered a suitable candidate for phytostabilization/ phytoremediation approach.

Introduction

Plants encounter environmental stressors throughout their life process (Bhat et al., 2019). We have seen in recent decades a dramatic worsening of the environmental contamination by heavy metals (HM) which are known for their toxicity and persistence (Jamla et al., 2021) and also are admitted as a significant barrier to reaching optimal agricultural production worldwide (Alsahli et al., 2021). Among them, we take into consideration Arsenic and Aluminium.

Arsenic is a poisonous generally found in the environment and organisms (Ackova, 2018). Both natural and anthropological activities cause the introduction of this element into terrestrial and aquatic environments (Jamla et al., 2021). To detect the impact of different As species, diverse physiological and biochemical parameters of different plant species have been elucidated (Dave et al., 2013). In fact, the phytotoxicity of arsenic to plants can be varied with concentration, soil properties and phosphorus content (Jamla et al., 2021). The immersion of Arsenate into plants causes damage to root membranes (Ackova, 2018). High arsenic levels alter the growth and development of halophytes (Dave et al., 2013).

Contrarily, Aluminium is beneficial and boosts plant growth in small quantities, however, it is not compulsory for all plants (Rahman & Upadhyaya, 2021). The Al effect in plants can be identified as death of apical tissues, curly leaf, getting roots thickener and brown. Al may also produce callose and lignin deposits on root tips and may cause an unbalance in the metal homeostasis and signal transduction path (Shetty et al., 2021). Therefore, these heavy metals drop the antioxidant pools and thus rise in ROS production (Hossain et al., 2012). Consequently, the latter gives rise to trouble in the various physiological

activity of plants and results in reduced growth and yield (Kumari et al., 2019). As a result, the plant's susceptibility to heavy metals is based on an associated network of physiological and molecular mechanisms which involve metal absorption and storage via linking to extracellular exudates and cell walls through various substances. The ions are complexed into cells, and general biochemical stress defence feedback such as the stimulation of antioxidative enzymes and changing plant metabolism allows proper functioning of the metabolic path and rapid restoration of harmed cell structures (Branco-Neves et al., 2017).

On the other hand, plants are exposed to another stress factor that can affect ecosystems, namely salinity, which is the main environmental element that deteriorates soils and reduces crop productivity. In Tunisia, for example, saline areas (like sebkha, chott… etc) are being altered by heavy metal pollution as a consequence of industrialization over the past few years and the dumping of waste into these zones. HM phytoremediation of salt-altered soils is a challenge because the appropriate plant species must be able to cope with both saline and polluting stresses (Zhou et al., 2019). Due to their spontaneous growth in soils known to be excessively salty, the use of halophyte species to remediate heavy metal polluted soils is of significant importance, particularly for its economic costs (kumari et al., 2019). Halophytes plants are grown in harsh environmental occurrences, such as lack of water, unsteady temperature, high salinity, and contaminated soil. The *Tamarix* genus develops in a wide range of natural territory marked by soils with varying salinity (Terronos et al., 2016). Few data are focused on determining the impact of metals on *T. gallica* growth and development (Moreno-Jiménez et al., 2009; Sghaier et al., 2019a; 2020; Bencherif et al., 2020).

Our previous studies Sghaier et al. (2015; 2016 and 2019b) focused on determining the effect of As and Al in some physiological parameters and also on determining the subcellular localization of these metals inside plants. Understanding the tolerance mechanisms that plants took up towards oxidative stress engendered by accumulated metal ions is the salient point to deepen the tolerance of plants to heavy metals and therefore its ability for the phytoremediation process. Taking all this into consideration, the present data are directed to investigate (i) the extent of Al and As lead to oxidative stress in *T. gallica* (ii) antioxidant responses to each metal, and (iii) the impact of As and Al under salt stress conditions. These objectives are followed by determining MDA content and also by examining the variation in proline and glycine betaine content.

Materials And Methods

Plant sampling and Experimental Setup

T.gallica was propagated by stem cuttings (5 cm) obtained from mother plants cultivated in the natural habitat of these species (sabkha of Ariana in Tunisia). Cuttings were grown in plastic pots containing a mixture of perlite and gravel substrate (2:1; v/v), then, were irrigated with non-saline tap water for a period of six weeks to induce a rooting (For more details see Sghaier et al., 2015; 2019). Later, young rooted cuttings were supplied by Hewitt nutritive solution (Hewitt, 1966) supplemented or not with NaCl (200

mM), three-time by a week for 90 days as described in Sghaier et al. (2015; 2019). Finally, plants were divided into groups of six plants:

a) Control plants

b) Al or As 200 µM;

c) Al or As 500 µM;

d) Al or As 800 µM;

e) NaCl 200 mM;

f) Al or As 200 µM + NaCl 200 mM;

g) Al or As 500 µM + NaCl 200 mM;

h) Al or As 800 µM + NaCl 200 mM.

The experiments were realized in a greenhouse under semi-controlled conditions with a natural photoperiod, mean temperature (night-day) of 20–30°C, and relative humidity between 60 and 90%.

Metal concentration

Fresh plant material was blended in HNO_3: H_2SO_4: $HClO_4$ (10:1:0.5; v/v/v) in Teflon vessels for 2 h 30 min at 110°C (see details in Sghaier et al., 2015; 2019) and then filtered and diluted with distilled water to 50 ml. The blanks, used to set the atomic absorption spectrometer to zero, were treated in the same way as described above.

Oxidative stress biomarkers

Leaves and roots were collected and immediately frozen in liquid N_2 and stored at -80°C. All enzymatic analyses were carried out at 4°C. For the extraction procedure, 500 mg of fresh leaves were added to12 ml of sodium phosphate buffer (50 mM, pH 7.6) with 0.1 mM Na-EDTA. The mixture underwent centrifugation at 8923 rpm for 20 min, at 4°C, and the supernatant was undertaken for enzymes assays.

Ascorbate peroxidase (APX) was determined as described by Tiryakioglu et al. (2006) by recording the drop in absorbance upon oxidation of ascorbate at 290 nm.

Guaiacol peroxidase (GPOX) was carried out by the method of Bergmeyer et al. (1974) by recording rise in absorbance upon the formation of guaiacol oxidation products at 470 nm (ε= ¼ 26.6 mM^{-1} cm^{-1}).

Superoxide dismutase (SOD) activity was assayed according to Marklund and Marklund (1974) by noticing the decline of pyrogallol at 325 nm. Proteins were set according to Bradford (1976).

MDA content

MDA content was determined according to Heath and Packer (1968), by using a mixture (20% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid (TBA)). The homogenate was extracted at 95°C for 30 min, followed by centrifugation at 3000 *g* for 5 min at 4°C. The absorbance of the supernatant was read at 532 and 600 nm in a Shimadzu UV-1603 spectrophotometer.

Osmo-compatible solutes

Free proline was extracted from blended samples in aqueous 3% sulphosalicylic acid, underwent centrifugation at 10,000 rpm for 15 min, and the proline in the supernatant was determined using ninhydrin according to Bates et al. (1973).

GB was determined as described by Grieve and Grattan (1983). After dilution with 2 N H_2SO_4 followed by dissolving the pellet (composed of periodide crystals) in 9 mL of 1,2-dichloroethane. The final step, was reading of the absorbance at 365 nm.

Statistical analysis

As the data missed normality and homogeneity, the statistical analysis was based on non-parametric tests, the Kruskal Wallis test was performed using Statistica software (Statasoft).

Results

Biomass production

Figure 1 presented the alteration of fresh weight (FW) in the whole plant (roots and shoots) depending on the HM concentrations. The reduction in fresh weight plants was more pronounced in As treatment than those treated with Al. Our data showed that all tested As concentrations declined FW significantly, both root and shoot biomass declined markedly at each As level. This drop is above 50% in the different organs when doses 500 and 800 µM were incorporated in the medium leading to a diminution in the plants' length without visible chlorosis altering leaves. It varied from 5962 to 1362 mg FW and from 2133 to 1377 mg FW in shoots and roots, respectively. Furthermore, the salt treatment led to a different morphological response in *T. gallica*. The biomass production significantly decreased at 500 and 800 µM As in roots. In contrast, the addition of NaCl to the medium did not have a significant impact on shoots at 200 and 500 µM As (Fig. 1).

In the same way, Plants treated with increasing Al concentrations showed a significant drop in FW. This reduction is around 40% compared to control plants. In other lines, no significant variation was detected in the root fresh FW of plants treated with increasing doses of aluminium. In salty medium, except to plant treated with 200 mM NaCl which showed a decline in FW, plants exposed to combined stress demonstrated no observable changes in fresh weight compared with those treated with Al only. As

observed above, roots demonstrated a similar behaviour as shown with plants treated by Al and control plants.

Aluminium and Arsenic accumulation

In Tamarix gallica, the results showed that the HM accumulation was influenced by the concentration of the metal in the irrigation solution. The content of As was around 52 and 50 µg.g^{-1}FW at 500 and 800 µM, respectively at the level of the aerial and root parts (Fig. 2). The addition of NaCl in the medium affected the concentration of As at the aerial parts, while for the roots, the addition of salt dd not reduce its content with the exception of the 800 µM As, where there was a decline estimated at 35%. The same effect was observed in the case of leaves, from a concentration of 500 µM.

The endogenous aluminium rate was correlated with the increasing concentration of aluminium in the nutrient solution (Fig. 2). Thus, the content of this ion, in the root tissues, was only 2.16 mg / g FW for a dose of 200 µM $AlCl_3$ and reached 3.2 and 5.4 mg.g^{-1} at 500 and 800 µM $AlCl_3$ respectively. Contrarily to the As, the addition of the salt in the medium enhanced metal uptake in roots and shoots (Fig. 2).

MDA content

the plants' subjection to different concentrations of arsenic for 90 days induced a significant (+ 100%) augmentation in MDA levels in *T. gallica* leaves compared to control plants. This rise was observed only at high concentrations of the metal ion (500 and 800 µM); however, at low doses (200 µM) there was no significant difference between control and untreated plants (Fig. 3). The values were about 10 and 14 mmol g^{-1} FW under As and NaCl exposure at 800 µM, respectively, compared with the controls (5 mmol g^{-1} FW). The salt added to the irrigation solution increased the MDA content for plants treated only with NaCl. It should be quoted that MDA production was higher for combined stress (As added to NaCl) than As alone (Fig. 3). Plants subjected to combined stress (As, NaCl) also showed an augmentation in membranous lipoperoxidation compared to plants treated only with salt, and the MDA content was multiple by a factor of 2.82 compared with control plants (Fig. 3).

After exposure to different concentrations of aluminium, an increase in MDA content was observed in *T. gallica* compared to control plants. This rise remained constant regardless of the concentration of Al applied. Thus, the production of MDA was increased twice compared to the control plants (Fig. 3). Irrigation of plants with Al in the presence of NaCl (200 mM) did not produce significant changes compared to control plants (Fig. 3).

Enzyme activities

the stressed individuals presented a variation in the enzyme activity. These differences were more certain in the GPx and APX activity (peroxidase activity) (Figs. 4 and 5). Our results show that the predominant anti-oxidant peroxidase in *T. gallica* turned out to be the APX in the shoots.

In *T. gallica*, the variation of the SOD activity following the poisoning of plants by Al showed that the latter had no obvious effect (Fig. 5). Indeed, the activity of this enzyme remained stable regardless of the concentration applied in the medium with the exception of the 500 µM dose where there was a slight rise. However, salt addition had an inhibitory effect on SOD activity in the 200 mM NaCl treated plants, which increased with Al doses in the nutrient solution. Elevations of 29%, 64% and 100% were recorded, respectively, at 200, 500 and 800 µM Al in the presence of salt (Fig. 5).

As stress obviously declined SOD activities regardless of the As stress extent (Fig. 4). In addition, SOD activity decreased more than 50% at 200 mM NaCl. Finally, SOD activities were strongly improved with increasing As doses, there was an enhancement activity, especially at 500 and 800 µM combined with salt.

There was a drop in GPX activity in the leaves of *T. gallica* under As stress at 200 µM. However, such effects on the activity of this enzyme were not recorded in the presence of 500 µM As, whereas at 800 µM As, the activity decreased to 50% compared to 200 µM (Fig. 4). Reduced activities of GPX were detected with NaCl addition under high-stress levels. Indeed, no difference was detected at the level of GPX activity after the application of increasing doses of Al (Fig. 5). On the other hand, the salt added to the culture medium caused a significant drop in GPX activity in plants subjected to 200 mM NaCl and a dramatic drop was with the increasing doses of Al in the medium.

Under As stress, there was a slight promotion in APX activity. This augmentation was strong up to 57% at 800 µM. Interestingly, the salt added to the medium did not alter the activity of the studied antioxidant as compared to the controls and there was a huge activity at a high level of As in combined stress (Fig. 4).

APX exhibited enhanced activities in individuals suffering from Al stress (Fig. 5). We noted an increase of 86%. This elevation remained constant regardless of the concentration of Al in the irrigation solution. However, APX activity diminished by the addition of the salt but recovered levels under higher concentrations of metals.

Proline and glycinebetaine

Comparing leaf proline content, except for the control which had small proline content, *T. gallica* exhibited stable concentrations among the treatments with only minor and non-significant fluctuations. The rising in content was observed in control compared to other As concentrations (Fig. 6).

The addition of the salt in the medium supplemented with As did not also modify proline trend. It was observed a huge rising in proline content as compared to the control whereas among treatment plants did not show any variation in their contents (Fig. 6).

In *T. gallica*, the exposure of plants to Al for 90 days induced an enhancement of the proline content. The effect was more pronounced for plants treated with a high concentration of aluminium. The proline level was increased twice compared to those of the control plants. Plants subjected to the combined stress (Al, NaCl) had a higher production of proline compared to those treated only by Al alone (Fig. 6). The

concentration at 800 µM in the presence of salt was amplified 3 fold to that of the plants untreated and 1.5 of the plants treated with 800 µM Al.

Regarding the glycine betaine concentration, no obvious differences were detected among treatments for plants treated with As (about 5 µg. g^{-1} FW) while *T. gallica* visualized a significant rise in this amino acid concentration, concomitant with adding the salt to the medium (Fig. 6). In fact, this rising was stronger at 500 and 800 µM As as compared to control plants and plants treated with salt no changes were induced in GB content by 200 mM NaCl.

The production of glycine betaine in plants subjected to aluminium seemed to be little affected by the presence of Al in the irrigation solution (Fig. 6). The glycine betaine content remained constant with the variation in the concentration of the metal in the culture medium except at 500 µM where a decrease was observed. Plants subjected to the combined stress (Al, NaCl) showed a higher production of betaine glycine than those treated only by Al alone.

Discussion

In this study, we tried to elucidate the biochemical response of *T.gallica* as a response to various stressors such as arsenic, aluminium, or combined stress (Al / As + 200 mM NaCl).

The accumulation of metal As or Al in *T.gallica* was described previously in our study (Sghaier et al., 2015; 2019) but here we discussed the accumulation of metal concerning fresh weight. As or Al accumulation in plant increased with metal concentration rising in the medium. This result suggested that *T. gallica* could absorb and accumulate high As or Al concentrations. 100% of plants exposed to 800 µM As(V) remained alive and As or Al concentrations in roots and shoots still increased till the end of treatment (90 days), demonstrating that the plant was not saturated with toxic ions and was able to accumulate more. However, the salt added in the medium decreased As uptake by the plant at 800 µM. Contrarily, Al showed an enhanced uptake by salt addition in the medium. the rising of Al in the salty medium might be attributed to the increased Al^{3+} uptake and its translocation in complexed form. Meanwhile, *T. gallica* is salt cedars and it could exclude toxic ions through special cells presented in the leaves by the salt bladders and those explained the diminution of As uptake. Salinity could modify the bioavailability of heavy metals in soil owing to the reduction of metal sorption from the soil and also the displacement of metals from the below-ground part to the above-ground parts of the plants (Wahla & Kirkham 2008). Most plants considered to be tolerant had mechanisms to keep much of their As load in the root (Leonardi et al., 2021). Salinity reduced Cd^{2+} absorption and translocation in *Kosteletzkya virginica* (Han et al., 2012), whereas Cd^{2+} translocation factor rose with the addition of NaCl in *Sesuvium portulacastrum* (Ghnaya et al., 2007).

Further, Shoots and roots growth were dramatically affected by arsenic stress alone or in combined stress. Reduction in growth could be provoked by water deficiency and nutrient disproportion. Indeed, the depression in root development under As treatments could be caused by water deficiency and nutrient

disproportion. The latter led to an inhibited division of endodermal cells provoked by the rise in doses of apoplastic As and which resulted in a decline in root length (Sarath et al., 2022). However, in Al stress alone, roots were unaffected by high Al concentrations while shoots seemed to be altered by the high metal extent which might lead to a variety of cellular toxic alterations regarding cell division, localization and expression of the nucleolar proteins (Zhang et al., 2014). Aluminium was linked to phosphorus (P) in a reduced utilizable and insoluble form giving rise to P deficiency for plant development. The combined stress enhanced the growth of the shoots and had no effect on root growth. Some salt cedars are known to colonize polluted soils (Conesa et al., 2006) and grow on contaminated soils without showing symptoms of intoxication (Manousaki et al., 2008), this was in line with our experiment which demonstrated that any sign of chlorosis or necrosis was detected after 3 months exposed to high concentrations of metals.

Moreover, it was reported that a least decreased shoot and root development of *Suaeda maritima* was observed under As exposure alone or combined treatments which hypothesized that As stress did not set serious impacts on nutrient metabolism (Panda et al., 2017). Nevertheless, exist genotypes where root development was unaffected even at very high Al doses, demonstrating that species varied in their Al stress reaction mechanisms at the cellular and tissue level (Bojórquez-Quintal et al., 2017). Further, one of the possible reasons for defunding the enhancement of plant growth provoked by Al was the promotion of nutrient absorption. In hyperaccumulator plants, Al could stimulate or had no impact on nutrient metabolism (Bojórquez-Quintal et al., 2017). Indeed, it had been reported that Al could stimulate channels and Mg transporters in Al-resistant plants (Bojórquez-Quintal et al., 2017).

Our funding suggested that severe metals stress alone engendered oxidative stress determined by the increment MDA content. The results obtained clearly pointed out the installation of membrane lipoperoxidation in *T. gallica* after exposition to different doses of As or Al which reflected the installation of a state of oxidative stress in the studied species. In addition, it also appeared that salt stress, in turn, stimulated membrane lipoperoxidation (Reginato et al., 2014). The MDA content varied depending on the metals type and doses added to the soil. The MDA content was higher in As stress than Al.

In the case of arsenic stress, the overproduction of membrane lipids might be due to arsenic-induced ROS. These could instantly attack the hydrogen atom of a methylene group next to an unsaturated carbon atom. As could also facilitate lipid peroxidation by disrupting the structure of the membrane (Akter et al., 2005). Parallel to our results, arsenic treatment induced the lipid peroxidation of the membrane in rice seedlings (Gaikwad et al., 2020). It was clear that both treatments damaged the membrane by increasing the MDA content. Plants subjected to salt stress were altered via osmotic stress, nutritional deficiency, ion toxicity, etc., inducing overproduction of ROS (Rahman et al., 2021). Raised ROS production was harmful but played a key role in the expression of stress response genes and also in the activation of the metal resistance approach (Sarath et al., 2022).

Al bound to membranes could instantly cause membrane stiffening and therefore might facilitate the catalysis of membrane peroxidation (Wang et al., 2015). Likewise, Shamsi et al. (2008) had shown that Al

treatment indirectly produced ROS which caused a severe imbalance including ROS production and antioxidant defence, resulting in increased lipid peroxidation via stimulation of MDA production (Shahnez et al., 2011). Recent studies demonstrated that some modifications in gene expression might participate in the alleviation of Al toxic effects. For instance, Wu et al. (2015) revealed that overexpression of OsPIN2 would impair the Al-activated formation of ROS and reduced lipid peroxidation (LPO) in rice roots.

In contrast, the constant or attenuated level of the malondialdehydes appeared to be a characteristic of salinity tolerant plants (Reginato et al., 2014). In agreement with our findings, several studies reported that both treatments As and salt had no impact on H_2O_2 levels. For instance, *Atriplex atacamensis* (Vromman et al., 2016), *Suaeda maritima* (Panda et al., 2017) and *Kosteletzkya pentacarpos* (Zhou et al., 2019).) This supports the hypothesis that salinity succoured plants to overcome HMs stress when salt had no impact like (Zn and Pb) or increased HMs accumulation in the aerial part (Zhou et al., 2019).

It's noteworthy that As led to inhibit SOD activity in *T. gallica* notably upon As exposure, the level of decline was variable for As stress alone or in combined stress. Similar results were obtained in an arsenate-tolerant *Holcus lanatus*, where declined activities of SOD were noticed at high levels of As subjection (Hartley-Whitaker et al., 2001). The reduction in growth was the result of an inadequate enzymatic activity to capture the ROS following high exposure to As.

Sharma et al. (2010) demonstrated that *Salicornia brachiata* could up-synthesis the activity of SOD towards heavy metals like Cd, Ni and As, whereas, at higher concentrations, SOD activity dropped which marked that the oxygen scavenging function of SOD was altered (Zhang et al., 2007). Vitoria et al. (2001) suggested that the activity of SOD decreased due to the metal ions linked to the active centre of the enzyme at elevated doses of heavy metals. In fact, the inactivation of enzymes by interfering with sulfhydryl groups, interaction with the enzyme-substrate complex, or protein active groups, and denaturation of the enzyme protein were the consequence of As exposure (Reboredo et al., 2021).

The supplemented NaCl diminished SOD activity under moderate and severe Cd stress suggesting that NaCl limited the conversion of O_2^- radicals to H_2O_2. Nevertheless, with rising doses of NaCl concentration, the POD activities increased and reduced under moderate and severe Cd stresses indicating the enhanced and inhibited scavenging processes of H_2O_2 (Wali et al., 2017). In *Suaeda maritima* subjected to individual effects of salinity, arsenic and mixed impact of As and salinity induced a drop of about 30% SOD activity as compared to untreated plants (Panda et al., 2017). Therefore, a decline in SOD activity under stress conditions designated its application in the sequestration of stress-induced $O_2^{\bullet-}$ radicals (Rangani et al., 2016). The activity of SOD in the leaves was unchanged at a lower Al concentration. Meanwhile, an augmentation was noted at 500 µM and a decline in SOD activity was marked at a high level of Al despite that the same activity as control was observed. The SOD activity was significantly hampered after exposure to the salt, however, an increase in SOD activity was recorded hereafter subjection to the combined stress Al and salt.

The augmentation in SOD activity was interrelated to the overproduction of ROS or the overexpression of genes encoding SOD (Israr et al., 2006). Otherwise, Martınez-Domınguez et al. (2010) reported that *Spartina densiflora* faced oxidative stress in its habitat and harmonized its antioxidative system depending on the level of metal pollution.

GPX activity was not altered by As treatment while the activity of this enzyme was hampered dropping to near to half under 800 µM As. The addition of the salt in the medium altered dramatically the enzyme's activity. However, a stimulation in GPX activity at low As concentrations combined with the salt was noticed followed by a decrease at a high level of metal combined with salt. Unchanged GPX activity was noted as a response to Al stress demonstrating that the GPX enzyme reached a high level for the trapping of H_2O_2 generated by Al stress (Panda et al., 2017). Meanwhile, a sharp reduction was observed in combined stress (Al + salt).

Other studies showed different behaviour in GPX activity as a response to Al stress. Kouki et al. (2021) reported that Al-induced a decrease in GPX activity. However, augmentation of GPX around 31% was observed in *Vigna trilobata (L.)* Verde after exposure to 6 mM Al (Arundhathi et al., 2016). This was similar to the results observed in *Helianthus annus* L. (Jouili et al., 2011) subjected to Al stress where there was stimulation in GPX activity. In the cell, the GPX enzyme s known as very sensitive toward metal and it had the ability to change its activity (Kouki et al., 2021).

According to our data, the APX activity was stimulated by 200 µM Al. APX was inhibited after subjection to the salt or 200 µM Al + 200Mm NaCl followed by stimulation at 500, 800 µM combined with salt. Also, there was a significant rise in the transcript extent of all APX encoding genes in rice after 8 hr subjection to 20 ppm Al (Rosa et al., 2010). it was noteworthy that APX and GPX showed simultaneous enhancement and inhibition, which had been shown to work together for As tolerance alone or combined with salt.

APX played a key role in the ROS direction in higher plants during stress and because of a higher APX affinity for H_2O_2 than GPX (Sofo et al., 2015; Anjum et al., 2016).

Additionally, Ben Amor et al. (2006) noticed an enhancement in APX activity under salt treatment by 20 days in *Cakile maritima.* In a previous study, APX activity was decreased under low concentrations of arsenic and decreased under high arsenic doses in the wheat seedlings (Li et al., 2007). Also, Shri et al. (2009) mentioned that arsenic treatment increased APX activity in rice plants.

More than that, the application of 200 µM As + 200 mM NaCl induced a 54% rise in POX activity compared to untreated plants highlighting POX's role in *Suaeda maritima*, antioxidative defence strategies (Demir et al., 2013). This result demonstrated that both treatments (As + salt) caused a generation of H_2O_2 which was trapped either by APX or POX, in order to preserve relevant H_2O_2 quantities for stress persuading (Panda et al., 2017). Indeed, APX was included in the ASH-GSH cycle through H_2O_2

reduction. Hsu and Kao (2007) reported that the pre-treatment of *Oryza sativa* with H_2O_2 induced an enhancement in APX activity and protected rice plants following subjection to Cd.

APX could be recognized from guaiacol peroxidase (GPX) in terms of physiological function. GPX had a crucial role in the biosynthesis of lignin and antioxidant defence by consuming H_2O_2. The activity of GPX varied considerably according to the plant species and the conditions (Foyer & Noctor, 2003).

Hampered peroxidase activity might be due to blockade of essential functional groups, the substitution of essential metals with trace elements, modification in protein structure or integrity, and disruption of signal transduction of antioxidant enzyme. Enhanced peroxidase activity was interconnected of the phytotoxic metal not linked to cell walls or stored in vacuoles (Hsu & Kao, 2007). Anjum et al. (2016) reported that plants were more tolerant to heavy metal stress through enhancement of APX activity.

The obtained results highlighted the complexity of the synergy between the different antioxidant enzymatic activities in order to regulate the antioxidant metabolism of plants.

Taken all together, the stimulation or deactivation of an enzyme in the defence system was based on metal/metalloid types, doses and extent of subjection and plant species. Under harsh stress conditions, a plant could be too fragile to activate adequate antioxidant enzymes to preserve itself. However, the low activity of the antioxidant enzyme might designate the weak stress which was demonstrated by unchanged MDA values among treatments in combined stress (As/ Al + salt) or under Al stress. Antioxidant enzymes were not the only way to take off the most reactive ROS, (HO·). Hence, assaying antioxidant molecule levels, e.g., proline and glycine betaine were required to have a complete scenario of the scavenging capacity (Kofronová et al., 2020).

In fact, to sustain the ionic equilibrium into vacuoles, the cytoplasm cumulates compatible solutes (Parida & Das, 2005). Among these compatible solutes, proline (Pro) and glycine betaine (GB) were found (Moghaieb et al., 2004).

The higher level of stress applied, the greater proline levels registered (Szabados & Savouré, 2010). Besides its function as osmoprotective, proline was responsible for strengthening the antioxidant system and fighting stress damage as a singlet oxygen deactivator (Lehmann et al., 2010) and hydroxyl radical scavenger (Lehmann et al., 2010). This suggested that this molecule could be accumulated in cells towards Cd, Cu, and other heavy metals (Lefévre et al., 2009).

Recently, a proline cycle was involved in the OH^- scan was proposed. In this reaction, the proline first caught OH^- by H-abstraction, then, a second H-abstraction, which also caught another OH^-, giving the P5C. through P5CR / NADPH enzyme activity, the P5C was recycled to proline (Signorelli et al., 2014). *Atriplex halimus* under Cd stress showed a twofold enhancement in proline content (Lefevre et al., 2009). Many metals tolerant species such as *Armeria maritima, Deschampsia cespitosa*, and *Silene vulgaris*, demonstrated high proline content (Sharma & Dietz, 2006).

Our findings boosted the study of Backor et al. (2004) who reported that proline accumulation was one strategy of heavy metal resistance in plants. Also, Shackira and Puthur (2017) signalled the antioxidant and osmoregulatory roles of proline in *A. ilicifolius* towards metal stress. Similarly, Pavlik et al. (2010) revealed that the rising in the proline extent of *Spinacia oleracea* was linked to arsenic stress.

Additionally, heavy metals might stimulate the formation of quaternary ammonium compounds like glycine betaine. GB accumulation protected thylakoids and conserved membrane integrity and preserved other cellular structures. It regulated the quaternary structures of complex proteins and saves chloroplasts and photosystem II (Moghaieb et al., 2004). This was due to its role in osmotic adjustment and limiting the hampered effect of stress resulting in accelerated restoration of harmed PSIIs (Siddiqui et al., 2010).

In Atriplex halimus Cd exposure induced an increase in osmotic adjustment and as consequence the formation of compatible sugars as glycine betaine (Levèvre et al., 2009). *T. gallica* showed an augmentation in the proline content after treatment with Al and As, whereas, no variation in GB content after exposure to different As and Al concentrations. The unchanged GB content could be related to not enhancing the expression of BADH, a key gene for glycine betaine biosynthesis (Wali et al., 2016). Indeed, a decline in free amino acid like glycine, cysteine and proline could be caused by the generation of stress-sensitive proteins as metallothioneins, phytochelatins (Sarath et al., 2022). Whereas, the exposure to 200 mM NaCl increased the synthesis of GB in Al stress and at high As concentrations. In line with our results, Wali et al. (2016) revealed an increase of about 20% of glycine betaine leaf accumulation after exposure to 200 mM NaCl. Osmotic adjustment in *Salicornia europaea* and *Suaeda maritima* was obtained with a significant rise in glycine betaine (Moghaieb et al., 2004).

Otherwise, low doses of salinity did not improve proline and GB levels in plants that were not imposed to heavy metals. The low concentration did not affect the water uptake of the plant confirming that altered water content proceed as one of the signals for osmolyte synthesis (Sarath et al., 2022). Indeed, the combined use of NaCl with As in *Chenopodium quinoa* improved the metal resistance of this plant. Similarly, the salt associated with $CdCl_2$ raised the Cd resistance ability of the halophyte *Bruguiera cylindrica* (Sruthi & Puthur, 2021). Obviously, both these examples boosted that the combined application of NaCl and metal stress in halophytes enhanced the metal resistance of the halophytes more than when it was administered individually (Sarath et al., 2022).

Conclusion

This was a new report on cross-tolerance mechanisms of salt with arsenic/ aluminium in the halophyte *Tamarix gallica*, and its phytoremediation abilities. In this study, *T. gallica* demonstrated its potential to grow under elevated concentrations of As/Al without noticing any visible symptoms of toxicity. This species showed a different behaviour to cope with as/ Al stress alone or combined with salt. Furthermore, *T. gallica* had an enzymatic antioxidant system that, together with non-enzymatic antioxidants, defended itself against cell damage. Additionally, other mechanisms could be related to the ability of *T. gallica* to

tolerate and grow at high concentrations of Al or As alone or combined with salt and further investigations need to be elucidated. Otherwise, *T. gallica* showed that salinity helped to cope with metals stress. The latter were characteristics of sebkha, the native habitat of this species, which was known as a saline area for dumping industrial waste and which made *T. gallica* a suitable candidate for phytostabilization/phytoremediation in these local zones.

Declarations

Authors contributions: DBS and BD carried out all the experiments, assured data analysis and DBS prepared the manuscript. IC and NS supervised the work and corrected the manuscript. All authors read and approved the final manuscript.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding and/or Competing interests

funding: This work was supported by the Foundation for Science and Technology (FCT) funding research in the Marine and Environmental Sciences Centre (MARE) through the project UID/MAR/042 92/2013

competing interest: The authors have no relevant financial or non-financial interests to disclose."

Compliance with ethical standards

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

References

1. Ackova, D.G. (2018). Heavy metals and their general toxicity on plants. Plant Science Today, 5(1),15–19. https://doi.https://dx.doi.org/10.14719/pst.2018.5.1.355
2. Akter, K.F., Owens, G., Davey, D.E., & Naidu, R. (2005). Arsenic speciation and toxicity in biological systems. Reviews of Environmental Contamination and Toxicology. 184, 97–149. https://doi:10.1007/0-387-27565-7_3.
3. Al-Mefarrej, A.H. (2013). Growth characteristics and some wood quality of *Tamarix aphylla* seedlings irrigated with primary treated wastewater under drought stress. Asian Journal of Plant Sciences, 12,109–118.
4. Alsahli AA, Bhat JA, Alyemeni MN, & Ahmad, P. (2021). Hydrogen Sulfide (H2S) Mitigates Arsenic (As)-Induced Toxicity in Pea (*Pisum sativum* L.) Plants by Regulating Osmoregulation, Antioxidant Defense System, Ascorbate Glutathione Cycle and Glyoxalase System. Journal of Plant Growth Regulation, 40, 2515–2531. doi:10.1007/s00344-020-10254-6
5. Anjum, N.A., Duarte, B., Caçador, I., Sleimi, N., Duarte, A.C., & Pereira, E. (2016). Biophysical and Biochemical Markers of Metal/Metalloid-Impacts in Salt Marsh Halophytes and Their Implications.

Frontiers in Environmental Science, 4,1–13.

6. Arundhathi, A., Marisamy, K., Duraipandian, M., Sevugaperumal, R., & Ramasubramanian, V. (2016). Comparison of the Metal Toxicity due to Aluminum and Barium on the Growth Attributes of *Vigna trilobata* (L.) Verde. Bioengineering and Bioscience, 4(4), 64–69. https://doi.org/10.13189/bb.2016.040403.
7. Backor, M., Fahselt, D., & Wu, C.T. (2004). Free Proline Content Is Positively Correlated with Copper Tolerance of the Lichen Photobiont *Trebouxia erici* (Chlorophyta). Plant Science, 167(1), 151–157. https://doi.org/10.1016/j.plantsci.2004.03.012.
8. Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205–207. https://doi.org/10.1007/BF00018060.
9. Ben Amor, N., Jimenez, A., Megdiche, W., Lundgvisit, M., Sevilla, F., & Abdelly, C. (2006). Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. Physiologia Plantarum, 126,446–456.
10. Bencherif, K., Trodi, F., Hamidi, M., Dalpè, Y., & Hadj-Sahraoui, A.L. (2020). Biological Overview and Adaptability Strategies of *Tamarix* Plants, *T. articulata and T. gallica* to Abiotic Stress. In: Giri B, Sharma MP (eds.) Plant Stress Biology. Springer, Singapore. https://doi.org/10.1007/978-981-15-9380-2_14
11. Bergmeyer, H.U., Gawehn, K., & Grassl, M. (1974). Enzymes as biochemical reagents. In: Bergmeyer HU (Ed.) Methods in enzymatic analysis. Academic Press, New York, pp. 515–517.
12. Bhat, J.A., Shivaraj, S.M., Singh, P., Navadagi, D.B., Tripathi, D.K., Dash, P.K., Solanke, A.U., Sonah, H., & Deshmukh, R. (2019). Role of Silicon in Mitigation of Heavy Metal Stresses in Crop Plants. Plants (Basel, Switzerland) 8,71. https://doi.org/10.3390/plants8030071.
13. Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1–2), 248–254. https://doi.org/10.1016/0003-2697 (76)90527–3.
14. Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., & Martínez-Estévez, M. (2017). Aluminum, a Friend or Foe of Higher Plants in Acid Soils. Frontiers in Plant Science, 8,1767. https://doi.org/10.3389/fpls.2017.01767
15. Branco-Neves, S., Soares, C., de Sousa, A., Martins, V., Azenha, M., Gerós, H., & Fidalgo, F. (2017). An efficient antioxidant system and heavy metal exclusion from leaves make *Solanum cheesmaniae* more tolerant to Cu than its cultivated counterpart. Food and Energy Security, 6, 123–133.
16. Cao, X., Ma, L.Q., & Tu, C. (2004). Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). Environmental Pollution, 128, 317–25. https://doi.org/10.1016/j.envpol.2003.09.018.
17. Conesa, H.M., Faz, A., & Arnaldos, R. (2006). Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena-La Unión mining district (SE Spain). Science of The Total Environment, 366(1), 1–11. https://doi.org/10.1016/j.scitotenv. 2005.12.008.

18. Dave, R., Tripathi, R.D., Dwivedi, S., Tripathi, P., Dixit, G., Sharma, Y.K., Trivedi, P.K., Corpas, F.J., Barroso, J.B., & Chakrabarty, D. (2013). Arsenate and arsenite exposure modulate antioxidants and amino acids in contrasting arsenic accumulating rice (*Oryza sativa* L.) genotypes. *Journal of Hazardous Materials*, 15, 1123–31. https://doi.org/10. 1016/ j. jhazmat. 2012.06.049.
19. Demir, E., Seckin, D.B., & Ozdener, Y. (2013). Biochemical Effects of Arsenic Stress In The Leaves of Halophyte *Cakile maritima* (Scop.) Plants Under Salinity. Fresenius Environmental Bulletin, 22(12), 3465–3473.
20. Foyer, C.H., &Noctor, G. (2003). Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum, 119, 355–364.
21. Gaikwad, D.J., Pal, A., & Mondal, S. (2020). Effect of Arsenic Toxicity on Chlorophyll Content and Antioxidative Enzymes Activities in Rice Seedlings. *International Journal of Environment and Climate Change*. 10(12), 411–421. https://doi.org/10.9734/IJECC/ 2020/v10i1230318.
22. Ghnaya T, Slama I, Messedi D, Grignon C, & Abdelly C (2007) Cd-induced growth reduction in the halophyte *Sesuvium portulacastrum* is significantly improved by NaCl. Journal of Plant Research, 120(2), 309–316. https://doi.org/10.1007/s10265-006-0042-3.
23. Grieve, C.M., & Grattan, S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds Plant and Soil volume, 70, 303–307. https://doi.org/10.1007/BF02374789.
24. Han, R.M., Lefèvre, I., Ruan, C.J., Qin, P., & Lutts, S. (2012a). NaCl differently interferes with Cd and Zn toxicities in the wetland halophyte species *Kosteletzkya virginica* (L.) Presl. Plant Growth Regulation volume, 68, 97–109. https://doi.org/10.1007/s10725-012-9697-z
25. Hartley-Whitaker, J., Ainsworth, G., Vooijs, R., Ten bookum, W., Schat, H., & Meharg, A.A., (2001). Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus*. Plant Physiology, 126, 299–306. https://doi.org/10.1104/pp.126.1.299
26. Heath, R.L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 125(1), 189–198. https://doi.org/10.1016/0003-9861 (68)90654-1
27. Hewitt, E.J. (1966). Sand and water culture methods used in the study of plant nutrition. Journal Association of Official Analytical Chemists, 49, 888–889. https://doi.org/10.1093/jaoac/49.4.888a.
28. Hossain, M.A., Piyatida, P., Teixeira da Silva, J.A., & Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany. 1–37. https://doi.org/10.1155/2012/872875
29. Hsu, Y.T., & Kao, C.H. (2007). Toxicity in leaves of rice exposed to cadmium is due to hydrogen peroxide accumulation. Plant and Soil, 298, 231–241. https://doi.org/10.1007/s11104-007-9357-7
30. Israr, M., Sahi, S., Datta, R., & Sarkar, D. (2006). Bioaccumulation and physiological effects of mercury in *Sesbania drummondii*. Chemosphere. 65, 591–598. https://doi.org/10.1016/j.chemosphere. 2006.02.016.

31. Jamla, M., Khare T., Joshi S., Patil S., Penna S., & Kumar, V. (2021). Omics approaches for understanding heavy metal responses and tolerance in plants. Current Plant Biology, 27, 100213 https://doi.org/10.1016/j.cpb.2021.100213.
32. Jouili, H., Bouazizi, H., & El ferjani, E. (2011). Plants peroxidases: Biomarkers of metallic stress. Acta Physiologiae Plantarum, 33, 2075–2082. https://doi.org/10.1007/s11738-011-0780-2.
33. Kofroňová, M., Hrdinová, A., Mašková, P., Tremlová, J., Soudek, P., Petrová, Š., Pinkas, D., & Lipavská, H. (2020). Multi-Component Antioxidative System and Robust Carbohydrate Status, the Essence of Plant Arsenic Tolerance. *Antioxidants*, 9, 283. https://doi.org/10.3390/antiox9040283.
34. Kouki, R., Ayachi, R., Ferreira, R., & Sleimi, N., 2021. Behavior of *Cucumis sativus* L. in presence of aluminum stress: Germination, plant growth, and antioxidant enzymes. Food and Science Nutrition, 00, 1–9. https://doi.org/10.1002/fsn3.2294
35. Kumari, A., Goyal, V., & Sheokand, S., 2019. Oxidative Stress and Antioxidant Defence Under Metal Toxicity in Halophytes. In: Hasanuzzaman M, Nahar K, Öztürk M (eds.) Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes. Springer, Singapore. https://doi.org/10.1007/978-981-13-3762-8_6.
36. Kumari, A., Sheokand, S., Pooja, Kumar, A., Mann, A., Kumar, N., Devi, S., Rani, B., Kumar, A., & Meena, B. L. (2019) Halophyte growth and physiology under metal toxicity. In: Hasanuzzaman M, Nahar K, Öztürk M (eds.) ecophysiology, abiotic stress responses and utilization of halophytes. (Pte Ltd, pp 83–113) Springer Nature Singapore
37. Lefevre, I., Marchal, G., Correal, E., Zanuzzi, A., & Lutts, S. (2009). Variation in response to heavy metals during vegetative growth in *Dorycnium pentaphyllum* Scop. Plant Growth Regulation, 59, 1–11. https://doi.org/10.1007/s10725-009-9382-z
38. Lehmann, S., Funck, D., Szabados, L., & Rentsch, D. (2010). Proline metabolism and transport in plant development. Amino Acids, 39, 949–62. doi: 10.1007/s00726-010-0525-3.
39. Leonardi, C., Toscano, V., & Genovese, C. (2021). Evaluation of cadmium and arsenic effects on wild and cultivated cardoon genotypes selected for metal phytoremediation and bioenergy purposes. Environmental Science and Pollution Research, 28, 55102–55115. https://doi.org/10.1007/s11356-021-14705-9.
40. Li, C., Feng, S., Shao, Y., Jiang, L., Lu, X., & Hou, X. (2007). Effects of arsenic on seed germination and physiological activities of wheat seedlings. Journal of Environmental Sciences, 19(6), 725–732.
41. Manousaki, E., Kadukova, J., Papadantonakis, N., & Kalogerakis, N. (2008). Phytoextraction and phytoexcretion of Cd by *Tamarix smyrnensis* growing on contaminated non saline and saline soils. Environmental Research, 106(3), 326–332. https://doi.org/10.1016/j.envres.2007.04.004
42. Marklund, S., & Marklund, G. (1974). Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47(3), 469–474. https://doi.org/10.1111/j.1432-1033. 1974.tb03714.x
43. Martınez Domınguez, D., C'ordoba Garcıa, F., Canalejo Raya, A., & Torronteras Santiago, R. (2010). Cadmium-induced oxidative stress and the response of the antioxidative defense system in *Spartina*

densiflora. Physiologia Plantarum, 139(3), 289–302. https://doi.org/10.1111/j.1399-3054.2010.01368.x.

44. Moghaieb, R.E., Saneoka, H., & Fujita, K. (2004). Shoot regeneration from GUS-transformed tomato (*Lycopersicon esculentum*) hairy root. Cellular & Molecular Biology Letters, 9,439–49.
45. Moreno-Jiménez, E., Esteban, E., Carpena-Ruiz, R.O., & Peñalosa, J.M. (2009). Induced phytotoxicity in Mediterranean shrubs *P. lentiscus* and *T. gallica* after arsenic or mercury accumulation. Ecotoxicology and Environmental Safety, 72(6),1781–1789. https://doi.org/10.1016/j.ecoenv.2009.04.022
46. Panda, A., Rangani, J., Kumari, A., & Parida, A.K. (2017). Efficient regulation of arsenic translocation to shoot tissue and modulation of phytochelatin levels and antioxidative defense system confers salinity and arsenic tolerance in the Halophyte *Suaeda maritima*, Environmental and Experimental Botany, 143,149–171. https://doi.org/10.1016/j.envexpbot.2017.09.007.
47. Parida, A.K., & Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60(3), 324–49. https://doi.org/10.1016/j.ecoenv.2004.06.010.
48. Pavlik, M., Pavlikova, D., Staszkova, L., Neuberg, M., Kaliszova, R., Szakova, J., & Tlustos, P. (2010). The effect of arsenic contamination on amino acids metabolism in *Spinacia oleracea* L. Ecotoxicology and Environmental Safety, 73(10),1309–1313. https://doi.org/10.1016/j.ecoenv.2010.07.008
49. Rahman, M., Rahman, K., & Sathi, K.S. (2021). Supplemental Selenium and Boron Mitigate Salt-Induced Oxidative Damages in *Glycine max* L. Plants, 10, 2224. https://doi.org/10.3390/plants10102224.
50. Rahman, R., & Upadhyaya, H. (2021). Aluminium Toxicity and Its Tolerance in Plant: A Review. Journal of Plant Biology, 64,101–121. https://doi.org/10.1007/s12374-020-09280-4.
51. Rangani, J., Parida, A.K., Panda, A., & Kumari, A. (2016). Coordinated changes in antioxidative enzymes protect the photosynthetic machinery from salinity induced oxidative damage and confer salt tolerance in an extreme halophyte *Salvadora persica* L. Frontiers in Plant Science, 7, 1–18.
52. Reboredo, F.H., Pelica, J., Lidon, F.C., Pessoa, M.F., Silva, M.M., Guerra, M., Leitão, R., & Ramalho, J.C. (2021). The Tolerance of *Eucalyptus globulus* to Soil Contamination with Arsenic. Plants, 10, 627. https://doi.org/10.3390/plants10040627.
53. Reginato, M.A., Castagna, A., Furlán, A., Castro, S., Ranieri, A., & Luna, V. (2014). Physiological responses of a halophytic shrub to salt stress by Na2SO4 and NaCl: oxidative damage and the role of polyphenols in antioxidant protection. AoB Plants, 6, plu042. doi:10.1093/aobpla/plu042.
54. Rosa, S.B., Caverzan, A., Teixeira, F.K., Lazzarotto, F., Silveira, J.A.G., Ferreira-Silva, S.L., Abreu-Neto, J., Margis, R., & Margis-Pinheiro, M. (2010). Cytosolic APx knockdown indicates an ambiguous redox response in rice. Phytochemistry, 71,548–558 https://doi.org/10.1016/j.phytochem.2010.01.003.
55. Sarath, N.G., Shackira, A.M., El-Serehy, H.A., Hefft, D.I., & Puthur, J.T. (2022). Phytostabilization of arsenic and associated physio-anatomical changes in *Acanthus ilicifolius* L. Environmental Pollution, 298, 118828, https://doi.org/10.1016/j.envpol.2022.118828.

56. Sghaier, D.B., Duarte, B., Bankaji, I., Caçador, I., & Sleimi, N. (2015). Growth, chlorophyll fluorescence and mineral nutrition in the halophyte *Tamarix gallica* cultivated in combined stress conditions: Arsenic and NaCl. Journal of Photochemistry and Photobiology B: Biology, 149,204–214. https://doi.org/10.1016/j.jphotobiol.2015.06.003
57. Sghaier, D.B., Pedro, S., Diniz, M.S., Duarte, B., Caçador, I., & Sleimi, N. (2016). Tissue localization and distribution of As and Al in the halophyte *Tamarix gallica* under controlled conditions. Frontiers in Marine Science, 3, 274.
58. Sghaier, D.B., Bankaji, I., Pedro, S., Caçador, I., & Sleimi, N. (2019). Photosynthetic Behaviour and mineral nutrition of *Tamarix gallica* cultivated under Aluminum and NaCl combined stress. Phyton, 88, 239–252. https://doi.org/10.32604/phyton.2019.06887
59. Sghaier, D.B., Pedro, S., Duarte, B., Caçador, I., & Sleimi, N. (2019). Arsenic tolerance mechanism in halophyte plant: the case of *Tamarix gallica.* In: Hasanuzzaman M, Shabala S, Fujita M (eds.) Halophytes and Climate Change: Adaptive Mechanisms and Potential Uses CABI. pp, 255–265. https://doi.org/10.1079/9781786394330.0255
60. Sghaier, D.B., Pedro, S., Duarte, B., Diniz, M.S., Caçador, I., & Sleimi, N. (2020). Arsenic Accumulation, Compartmentation, and Complexation in *Arthrocnemum indicum*. In: Hasanuzzaman M (ed) Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives I springer. https://doi.org/10.1007/978-981-15-2156-0_25
61. Shackira, A.M., & Puthur, J.T. (2017). Enhanced phytostabilization of cadmium by a halophyte-*Acanthus ilicifolius* L. International Journal of Phytoremediation, 19(4), 319–326. https://doi.org/10.1080/15226514.2016.1225284.
62. Shahnaz, G., Shekoofeh, E., Kourosh, D., & Moohamadbagher, B. (2011). Interactive effects of silicon and aluminum on the malondialdehyde (MDA), proline, protein and phenolic compounds in *Borago officinalis* L. Journal of Medicinal Plants Research, 5(24), 5818–5827.
63. Shamsi, I.H., Wei, K., Zhang, G.P., Jilani, GH., & Hassan, M.J. (2008). Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. Biologia Plantarum, 52, 165–169. https://doi.org/10.1007/s10535-008-0036-1.
64. Sharma, A., Gontia, I., Agarwal, P.K., & Jha, B. (2010). Accumulation of heavy metals and its biochemical responses in *Salicornia brachiata*, an extreme halophyte. Marine Biology Research, 6(5), 511–518. https://doi.org/10.1080/17451000903434064.
65. Sharma, S.S., & Dietz, K.J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany, 57(4), 711–26. https://doi.org/10.1093/jxb/erj073.
66. Shetty, R., Vidya, C.S.N., Prakash, N.B., Lux, A., & Vaculík, M. (2021). Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. Science of the Total Environment, 765, 142744. https://doi.org/10.1016/j.scitotenv.2020.142744.
67. Shri, M., Kumar, S., Chakrabarty, D., Triverdi, P.K., Mallick, S., Misra, P., Shukla, D., Mishra, S., Srivastava, S., Tripathi, R.D., & Tuli, R. (2009). Effect of arsenic on growth, oxidative stress, and

antioxidant system in rice seedlings. Ecotoxicology and Environmental Safety, 72(4),1102–1110. https://doi.org/10.1016/j.ecoenv.2008.09.022.

68. Sruthi, P., & Puthur, J.T. (2021). Cadmium stress alleviation potential of *Bruguiera cylindrica* (L.) Blume enhances in combination with NaCl. Bioremediation Journal, 26(2), 1–24. https://doi.org/10.1080/10889868.2021.1911923.
69. Siddiqui, M.H., Mohammad, F., Khan, M.N., Al-Whaibi, M.H., & Bahkali, A.H.A. (2010). Nitrogen in relation to photosynthetic capacity and accumulation of osmoprotectant and nutrients in Brassica genotypes grown under salt stress. Agricultural Sciences in China, 9(5):671–680. https://doi.org/10.1016/S1671-2927(09)60142-5
70. Signorelli, S., Coitiño, E.L., Borsani, O., & Monza, J. (2014). Molecular mechanisms for the reaction between (˙) OH radicals and proline: insights on the role as reactive oxygen species scavenger in plant stress. Journal of Physical Chemistry B, 118(1), 37–47. https://doi.org/10.1021/ jp 407773u.
71. Sofo, A., Scopa, A., Nuzzaci, M., & Vitti, A. (2015). Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. International Journal of Molecular Sciences, 16(6),13561–13578. https://doi.org/10.3390/ijms160613561
72. Stolt, J.P., Sneller, F.E.C., Bryngelson, T., Lundborg, T., & Schat, H. (2003). Phytochelatin and cadmium accumulation in wheat. Environmental and Experimental Botany, 49(1), 21–28. https://doi.org/10.1016/S0098-8472(02)00045-X
73. Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Sciences*, 15(2), 89–97. https://doi.10.1016/j.tplants.2009.11.009.
74. Terrones, A., Moreno, J., Agulló, J.C., Villar, J.L., Vicente, A.M., Alonso, Á., & Juan, A. (2016). Influence of salinity and storage on germination of *Tamarix* taxa with contrasted ecological requirements. Journal of Arid Environments, 135, 17–21. https://doi.org/10.1016/j. jaridenv.2016.08.001.
75. Tiryakioglu, M., Eker, S., Ozkutlu, F., Husted, S., & Cakmak, I. (2006). Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. Journal of Trace Elements in Medicine and Biology, 20(3), 181–189 https://doi.org/10.1016/j.jtemb.2005.12. 004.
76. Vitória, A.P., Lea, P.J., & Azevedo, R.A. (2001). Antioxidant enzymes responses to cadmium in radish tissues. Phytochemistry,57,701 – 10 https://doi.org/10.1016/s0031-9422(01)00130-3.
77. Vromman, D., Lefèvre, I., Šlejkovec, Z., Martínez, J.P., Vanhecke, N., Briceño, M., Kumar, M., & Lutts, S. (2016). Salinity influences arsenic resistance in the xerohalophyte *Atriplex atacamensis* Phil. *Environmental and Experimental Botany*, 126? 32–43. https://doi.org/10.1016/j.envexpbot. 2016.01.004
78. Wahla, I.H., & Kirkham, M.B. (2008). Heavy metal displacement in salt-water-irrigated soil during phytoremediation. Environmental Pollution, 155, 271–83. https://doi.org/10.1016/j.envpol. 2007.11.020.
79. Wali, M., Gunsè, B., Lugany, M., Corrales, I., Abdelly, C., Poschenrieder, C., & Ghnaya, T., 2016. High salinity helps the halophyte *Sesuvium portulacastrum* in defense against Cd toxicity by maintaining redox balance and photosynthesis. Planta, 244, 333–346.

80. Wali, M., Martos, S., Pérez-Martín, L., Abdelly, C., Ghnaya, T., Poschenrieder, C., & Gunsé, B. (2017). Cadmium hampers salt tolerance of *Sesuvium portulacastrum*. Plant Physiology and Biochemistry, 115, 390–399. https://doi.org/10.1016/j.plaphy.2017.04.014
81. Wang, L., Fan, X.W., Pan, J.L., Huang. Z.B., & Li. Y.Z. (2015). Physiological characterization of maize tolerance to low dose of aluminum, highlighted by promoted leaf growth. Planta, 242,1391–1403 https://doi.org/10.1007/s00425-015-2376-3.
82. Wu, D., Shen, H., Yokawa, K., & Baluška, F. (2015). Overexpressing OsPIN2 enhances aluminium internalization by elevating vesicular trafficking in rice root apex. Journal of Experimental Botany, 66(21),6791–6801. https://doi.org/10.1093/jxb/erv385.
83. Zhang, F.Q., Wang, Y.S., Lou, Z.P. & Dong, J.D. (2007). Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel and Bruguiera gymnorrhiza*). Chemosphere, 67, 44–50 https://doi.org/10.1016/j.chemosphere.2006.10.007
84. Zhang, H., Jiang, Z., Qin, R., Zhang, H., Zou, J., Jiang, W., & Liu, D. (2014). Accumulation and cellular toxicity of aluminum in seedling of *Pinus massoniana*. BMC Plant Biology, 14, 264. https://doi.org/10.1186/s12870-014-0264-9
85. Zhou, M., Engelmann, T., & Lutts, S. (2019). Salinity modifies heavy metals and arsenic absorption by the halophyte plant species *Kosteletzkya pentacarpos* and pollutant leaching from a polycontaminated substrate. Ecotoxicology and Environmental Safety, 182, 109460. https://doi.org/10.1016/j. ecoenv.2019.109460.

Figures

Fig. 1

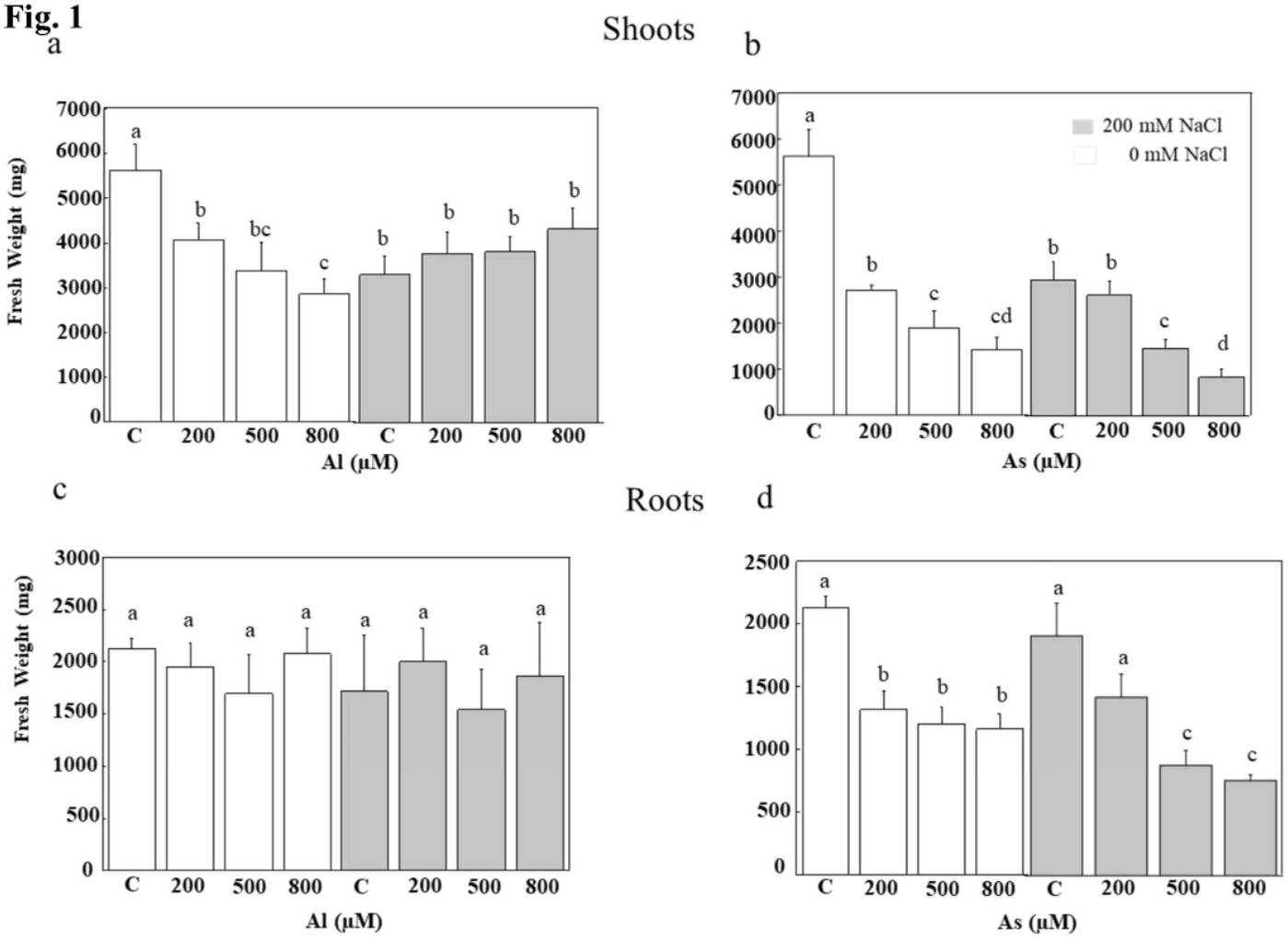

Figure 1

Biomass production in shoots (a, b) and roots (c, d) tissues of *Tamarix gallica* under different concentrations of As or Al (0,200,500, 800 μM) in the absence or presence of 200 mM NaCl in the nutrient solution (mean values ±SD, n=3). Different lower-case letters represent statistically significant differences ($p<0.05$)

Fig. 2

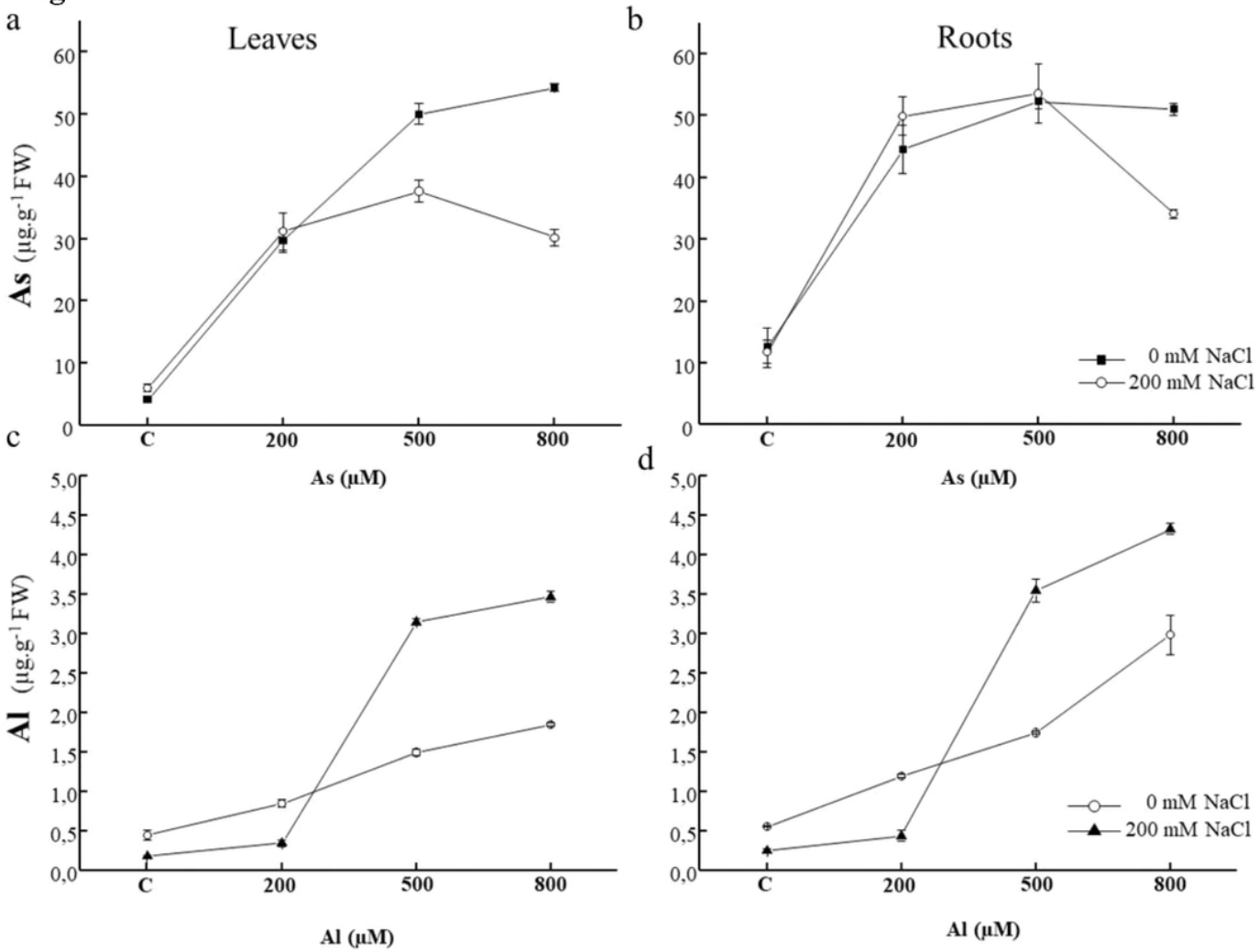

Figure 2

Arsenic (a, b) and Al (c, d) concentrations in shoots and roots tissues of *Tamarix gallica* (mean ±SD, n=3) exposed to different doses (0,200,500, 800 µM) of As or Al in the absence or presence of 200 mM NaCl in the nutrient solution.

Fig. 3

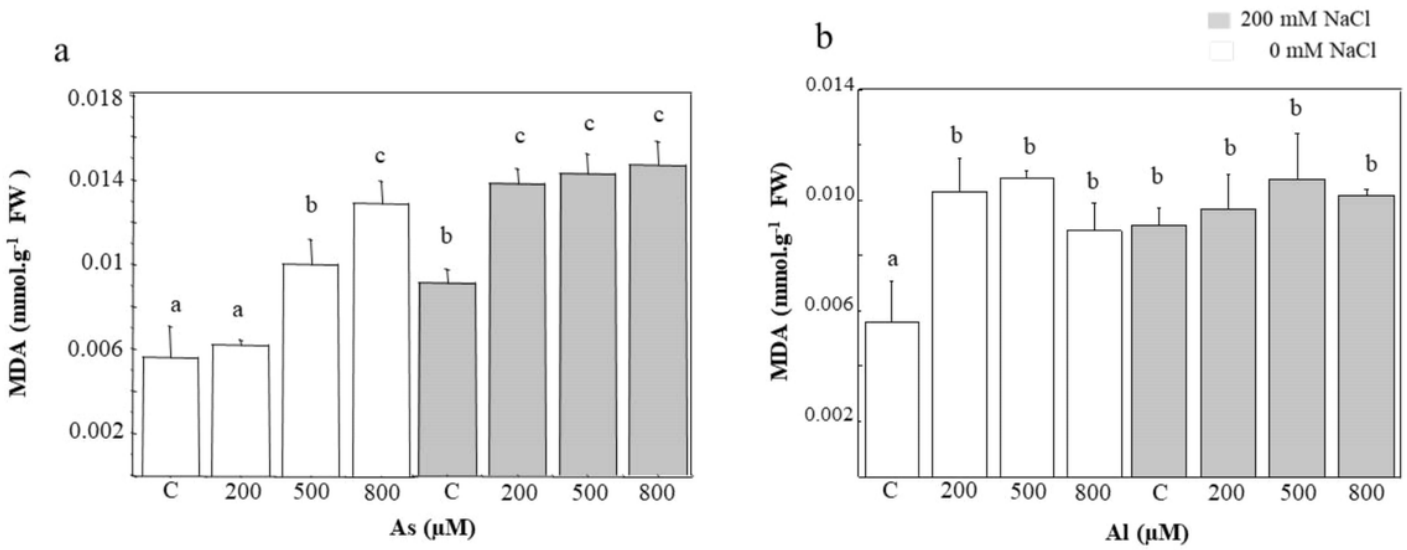

Figure 3

MDA content in the leaves of *Tamarix gallica* under different concentrations (0,200,500, 800 µM) of As (a) or Al (b) in the absence or presence of 200 mM NaCl in the nutrient solution (mean values ±SD, n=3). Different lower-case letters represent statistically significant differences ($p<0.05$)

Fig. 4

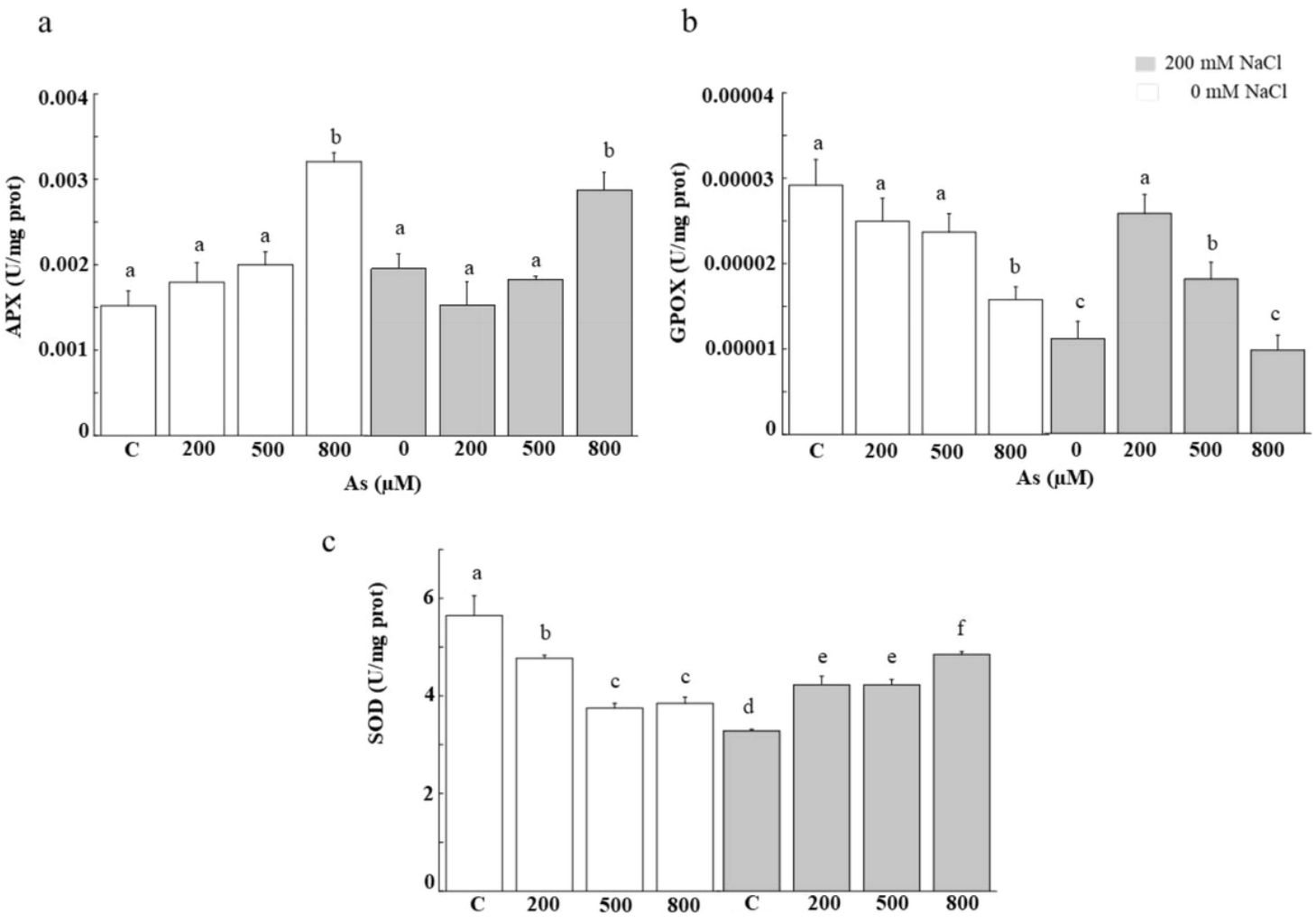

Figure 4

Enzymatic activity (APX (a), GPX (b) and SOD (c) in the leaves of *Tamarix gallica* (mean values ±SD) under different concentrations of As (0,200,500, 800 μM) in the absence or presence of 200 mM NaCl in the nutrient solution (mean values ±SD, n=3). Different lower-case letters represent statistically significant differences ($p<0.05$)

Fig. 5

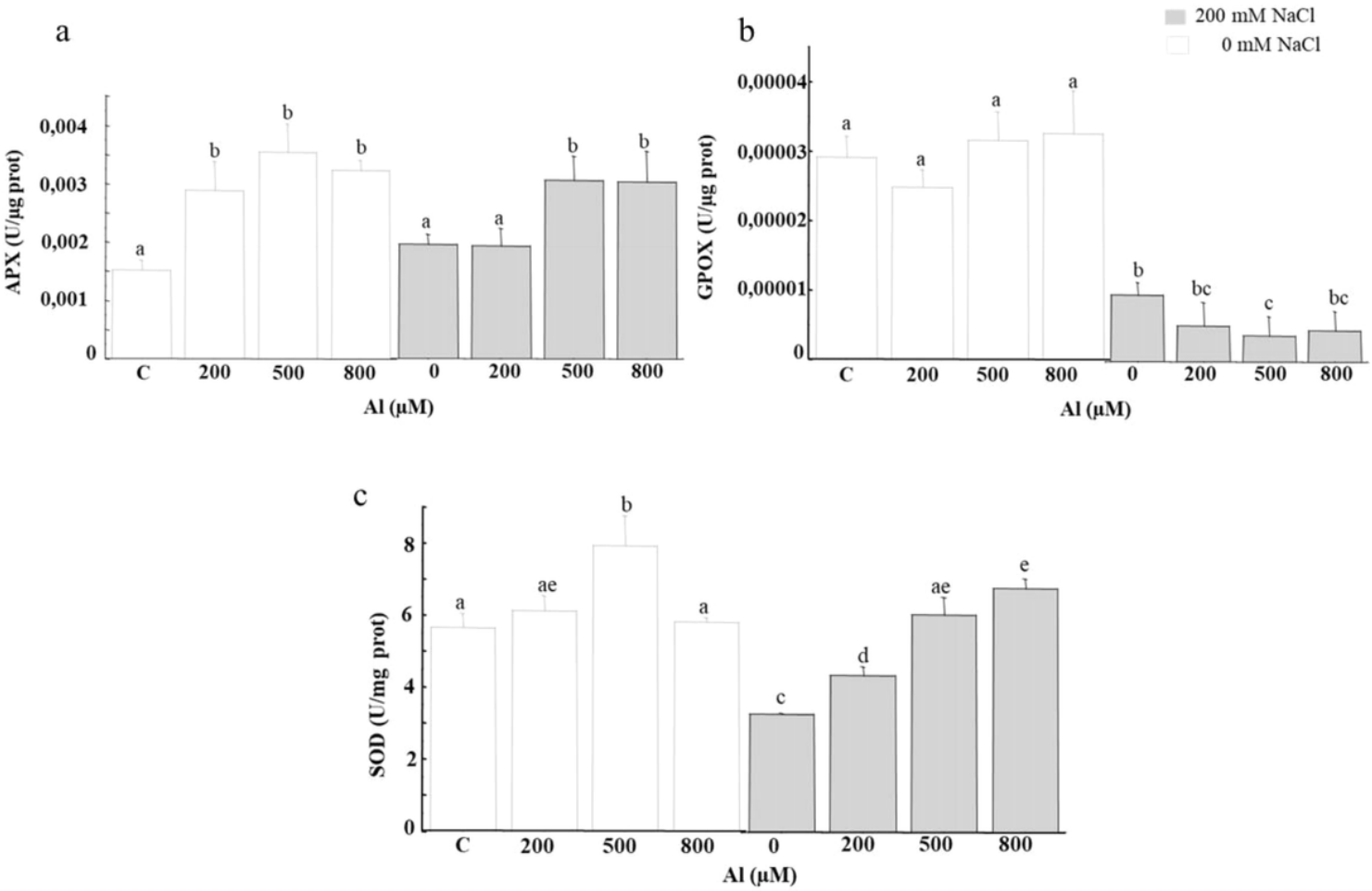

Figure 5

Enzymatic activity (APX (a), GPX (b) and SOD (c) in the leaves of *Tamarix gallica* under different concentrations of Al (0,200,500, 800 µM) in the absence or presence of 200 mM NaCl in the nutrient solution (mean values ±SD, n=3). Different lower-case letters represent statistically significant differences ($p<0.05$)

Fig. 6

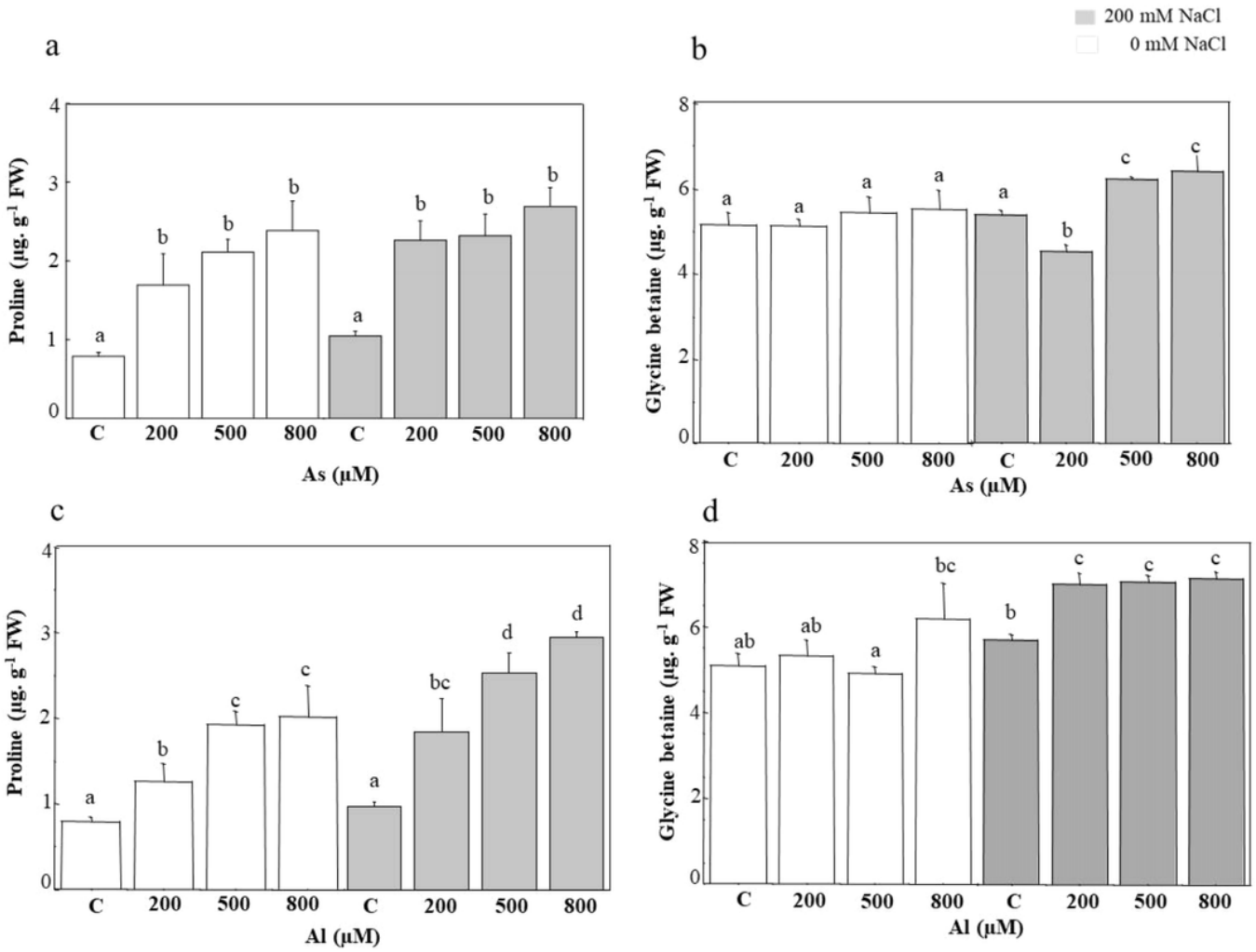

Figure 6

Proline and glycine betaine content in the leaves of *Tamarix gallica* under different concentrations (0,200,500, 800 μM) of As (a, c) or Al (b, d) in the absence or presence of 200 mM NaCl in the nutrient solution (mean values ±SD, n=3). Different lower-case letters represent statistically significant differences ($p<0.05$)