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Lead tolerance and accumulation in *Arthrocaulon macrostachyum*: Growth, physiological and antioxidant responses

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ABSTRACT

Lead (Pb) is a non-essential and toxic metal for plants. Current study was planned to assess Pb-induced toxicity with and without NaCl in a halophyte *Arthrocaulon macrostachyum*. Three month old seedlings (after germination) were grown in pots containing quartz sand and sub-irrigated with 300 mM NaCl and different Pb$(NO_3)_2$ concentrations (0, 0.75, 3, 6 and 12 mM) in plastic trays where 0 mM NaCl and Pb$(NO_3)_2$ were taken as control for monitoring growth, physiological and biochemical characteristics. Plant growth (height and biomass) was optimized in 300 mM NaCl while progressive increases in fresh weights up to 3 mM Pb$(NO_3)_2$ may be related to better shoot turgor, succulence and more plant pigments. Growth was substantially reduced in 6 and 12 mM Pb$(NO_3)_2$ both with and without NaCl. Plant shoots accumulated higher levels of Pb and Na$^+$ of up to 3 mM Pb;while root Pb increased in $>$ 6 mM Pb$(NO_3)_2$ treatments. Decreased pigments, potential quantum yield (Fv/Fm) and yield of photosystem II (ϕ PSII) enhanced the accumulation of H_2O_2, electrolyte leakage and consequently reactive oxygen species (ROS) in 12 mM Pb$(NO_3)_2$. The scavenging of ROS was manifested with the increased *in vitro* activity of superoxide dismutase (SOD) of up to 6 mM Pb$(NO_3)_2$; whereas higher Guaiacol peroxidase (GPx) may be related to cell wall hardening and poor growth. The increases in SOD, marginal changes in catalase (CAT) and unchanged APX of up to 6 mM Pb$(NO_3)_2$ demonstrated the activation of stress-responsive metabolism in *Arthrocaulon machrostachyum*; and Pb accumulation of up to 200 mg kg^{-1} making this species a useful candidate for Pb phytoremediation.

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1. Introduction

Trace metal pollution and soil salinization have augmented land degradation at the global level (Munns and Gilliham, 2015; Rodríguez-Eugenio et al., 2018; Schück and Greger, 2023). In addition to the climate changes, increasing anthropogenic activities such as the use of excessive chemicals in agriculture, industrial as well as urban discharges have contributed to a rise in pollution which has ultimately resulted in biodiversity loss (Mujeeb et al., 2021; Rahman et al., 2024; Van Oosten and Maggio, 2015). Release of non-biodegradable trace metals due to pollution consequently have a negative impact on ecosystems and environment and eventually toxic for the life on earth (Mujeeb et al., 2020; Rosado et al., 2016). Trace metal toxicity not only hampers plant growth but also deteriorates human health via their entry through food chain (Alamgir et al., 2024; Rehman et al., 2017; Shaghaleh et al., 2024; Tan et al., 2010).

Although plants essentially utilize some trace metals for growth e.g., zinc, iron, copper, manganese etc. (de Bang et al., 2021; Jalil et al., 2023; Taiz and Zeiger, 2015), others require lead, chromium, arsenic etc., have no or little role as minerals both for plants and animals (Li et al., 2024; Qureshi et al., 2024; Yong et al., 2010). Non-essential metals at elevated levels disturb physiology and may damage enzymatic machinery (Ekmekci et al., 2009) therefore, they need to be removed from environment by using different methods (Khilji et al., 2022; Lutts and Lefèvre, 2015; Qureshi et al., 2024; Schück and Greger, 2023; Shi et al., 2018; Singh et al., 2022; Tow et al., 2018; Yong et al., 2010). Plants that may resist metal toxicity without any visible symptoms are called “metallophytes” and these halophytes are no different as they have the enhanced biological ability to remove higher levels of metals (Christofilopoulos et al., 2016; Korzeniowska and Stanislawska- Glubiak, 2015; Lutts and Lefervre, 2015; Manousaki et al., 2014; Mujeeb et al., 2021; Mujeeb et al., 2020; Nikalje and Suprasanna, 2018). These indigenous plants of the wild can be explicitly used for removing toxic metal ions by keeping tight physiological regulation (Lutts and Lefèvre, 2015; Manousaki and Kalogerakis, 2011; Munns et al., 2019).

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Among heavy metals, cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) are recognized as main pollutants with no beneficial effects on plants (Bernard, 2008; Li et al., 2024; Liu et al., 2012; Swathika et al., 2024). Of these metals, Pb is hazardous and ubiquitous contaminant that is released from industries and use of commercial fertilizers and other chemicals due to which it may persist in soil environment for longer periods (Haghighizadeh et al. 2024). The recommended ranges of Pb in soils are 300–600 mg kg^{-1} (Ashraf et al., 2019), however, even the low concentrations in soils and irrigation water may be harmful for different organisms and disturbs the ecological equilibrium (Shi et al., 2018). Lead (Pb) as non-essential element can damage membrane bound organelles, distort enzymatic activities due to ROS production, reduces seed germination, mineral uptake, and overall growth of plants besides stimulating the synthesis of H_2O_2 (Sharma and Dubey, 2005; Zhao et al., 2018). Interestingly, some species can survive in soils containing high Pb concentrations (no visible phytotoxicity symptoms) and hyperaccumulate Pb at levels between 300 and 1000 mg kg^{-1} in their dried shoots (Ashraf et al., 2019).

Adaptations to elevated levels of salts and metals in halophytes is achieved via osmo-tolerance, water balance and enhancing antioxidant systems (Amari et al., 2017; Munns et al., 2019; Patel and Parida, 2021; Wu et al., 2023). Plants that are sensitive to minor trace element concentrations often undergo reduced primary root elongation, secondary growth, root dieback that usually disrupt root-absorbing area which could hamper water uptake and mineral acquisition (Rucinska-Sobkowiak, 2016; Ningombam et al. 2024). Halophytes can avoid metal ions spatially at the cellular level through compartmentalization by either in the vacuole or apoplast; while at shoot level, they can resist metals by producing metallothioneins (MT's) (Aziz and Mujeeb, 2022). Some of the halophytes can tolerate waterlogging, salinity and contaminants e.g., *Aegiceras corniculatum, Avicennia marina, Aster tripolium, Atriplex nummularia, Halimione portulacoides, Mesembryanthemum crystallinum, Tamarix smyrnensis,* and *Sesuvium portulacastrum* (Aziz and Mujeeb, 2022; Lokhande and Suprasanna 2012). Besides other morpho-anatomical features, halophytes have evolved special adaptive mechanisms to deal with multiple stress factors simultaneously (Suzuki et al., 2014), such as selective ion absorption from the soil, secretion of toxic ions like Na^+ through leaf surface (via trichomes or glands) and vacuolar compartmentalization (Jiang and Liu, 2010; Schück and Greger, 2023).

Arthrocaulon macrostachyum (synonym *Arthrocnemum macrostachyum*) is a C_3 stem succulent halophyte and a known bio-indicator of Cd, Pb, As, and others (Martínez-Sánchez et al., 2012). Bioindicators accumulate metals in above-ground parts while maintaining strong linear correlations with soil metals (Aziz and Mujeeb, 2022; Van der Ent et al., 2013). *Arthrocaulon macrostachyum* can also tolerate extreme salinities of ~1 M (1000 mM) NaCl or higher (Khan et al. 2005) and an important non-conventional crop for agricultural countries with warm saline coasts (Paraskevopoulou et al., 2015). It is found all along the Mediterranean coast, Macaronesia and parts of Africa, particularly Angola (southern part of Africa) and Arabian Peninsula (Gairola et al., 2015). The species is known for soil desalinization (Jurado et al. 2024) and avoidance from toxic metals for example Cd, As, Pb and Cu (Martinez-Sanchez et al., 2012; Gairola et al., 2015). Although, mechanisms of salinity resistance and presence of different metals in *A. macrostachyum* plants are explained in earlier studies by Martinez-Sanchez et al. (2012), information regarding Pb and NaCl tolerance as well as their extent of translocation to aerial parts is largely unexplored. Field studies on *A. macrostachyum* suggest that the bioavailability of Pb changes with seasonal fluctuations (Mujeeb et al., 2021). Therefore, it would be interesting to check Pb translocation to shoots in *A. macrostachyum* under saline conditions since the species is known for excreting metal ions (Usman et al., 2019).

In Pakistan, *Arthrocaulon macrostachyum* is found on the coastal salt marshes of Karachi and Balochistan (Khan et al. 2005). This plant can also withstand waterlogged conditions (Jurado et al., 2024) and can remove trace metals (Martinez-Sanchez et al., 2012). Therefore, using such important species for cleaning saline and waterlogged polluted soils would help in land reclamation particularly for the standing crops. Since the underlying mechanisms of tolerance in *A. macrostachyum* to NaCl and extraction ability of Pb is largely unknown, current study was aimed at evaluating its physiological and biochemical responses. (Mujeeb et al. 2021) reported in earlier field studies that Pb is a prevalent metal which is accumulated in tissues of most of the coastal plants. Therefore, variable concentrations of $Pb(NO_3)_2$ were tested, with and without NaCl, for growth and biochemical parameters of *A. macrostachyum*. Bioaccumulation factor (BAF) as translocation (TF) in shoots was also calculated to monitor the level of metal accumulation in tissues. The following questions were addressed in the current research work: 1) What is the level of Pb tolerance in *Arthrocaulon macrostachyum* under saline conditions? 2) Is Pb and NaCl tolerance in *Arthrocaulon macrostachyum* related to oxidative stress management and other adaptations?

2. Materials and methods

2.1. Material collection and growth conditions

Arthrocaulon macrostachyum seeds were obtained from the marshes of coastal area near sand-spit (24.83190746811807, 66.92183071374966). Seeds were germinated in ambient environmental conditions in a green netted cage (Ave temp: 32 °C; 12-h day light ~ 400 μmol m^{-2} s^{-1}) with mixture of sandy soil and organic manure (1:1). After three months of seedling establishment with 5 to 6 nodes, plants were shifted into open cage (~ 1200 μmol m^{-2} s^{-1} day light: min: 990 and max: 1300 and Ave temp: 32; min 26 and max: 37 C) and transplanted to industrial quartz sand (~0.5 kg) in plastic containers (volume ~ 950 cm^3) which filled approximately 3/4th of the pots. Prior to transferring plants, quartz sand was thoroughly washed and dried. Subirrigation applies water below the soil surface to raise the water table into or near the plant root zone.

2.2. Selection of Pb concentrations for growth experiments

After transplanting *A. macrostachyum* into quartz sand, 3 pots (one plant in each pot) were placed in each tray containing 2 l of half strength Hoagland nutrient solution (Epstein, 1972). This sub-irrigation technique was used to raise water table near the plant root zone. Considering the levels of Pb up to 1000 mg kg^{-1} in soils near the industries (Pourrut et al., 2011), following concentrations of $Pb(NO_3)_2$ *viz*., 0 mM (Control), 0.75 mM (250 mg kg^{-1}) and 3 mM (1000 mg kg^{-1}) were used. However, no significant change in plant biomass and height was found even after 2 weeks hence the concentrations of $Pb(NO_3)_2$ were further raised to 6 ad 12 mM (Pb $(NO_3)_2$ doses (corresponding to 2000 and 4000 mg kg^{-1}). Hence, after reassessing initial trials [i.e., 0, 0.75 and 3 mM $Pb(NO_3)_2$], five doses each of Pb [0, 0.75, 3, 6 and 12 mM Pb $(NO_3)_2$] with and without 300 mM NaCl (Supplementary Fig. 1) with 6 replicates each (10 × 6 = 60) were selected for final experiment. The dosage of 0.75 mM $Pb(NO_3)_2$ (equal to 250 mg kg^{-1}) per day was incremented in each treatment until the final treatment of 12 mM $Pb(NO_3)_2$ was reached on 8th day. Trays were arranged in a completely randomized design (CRD). The entire experiment was continued for 4 weeks after the final treatment (12 mM) was achieved. The treatment solutions in sub-irrigation trays were completely changed once in every week. However, due to diurnal evaporative losses, water in each tray was added daily to maintain 2-liter quantity of nutrient solutions.

2.3. Growth parameters

Plants of *A. macrostachyum* were harvested for growth parameters after 4 weeks (*viz*., root and shoot lengths, total plant height and number of shoot branches). Plant roots and shoots were carefully separated, cleaned via sonication (use of ultrasonic waves), and weighed immediately on a precision balance. Sonication of plant samples was done to remove any dirt of soil particles using a Branson 2510 Sonicator (Marsall Scientific). All plant samples were then dried in a heating oven (Thermo-scientific, USA) for 2–3 days at 60 °C or when constant dry weights (DW) were obtained.

2.4. Water relations

Shoot water and osmotic as well as turgor potential in *A. macrostachyum* were determined a day before the final harvest. Water potential was determined psychrometrically on shoots with the help of Dew point microvoltmeter (Wescor, Inc., Logan, USA) and values were recorded. All values were changed into megapascals (-MPa) using regression equation plotted from the standard curve with NaCl. Osmolality on press saps of same shoots were determined using Osmometer (Vapro-5520, Wescor, Inc. USA) and values converted into megapascal (-MPa) by applying Vant Hoff's equation. Turgor potential was calculated by subtracting osmotic potential from water potential (Shoukat et al., 2018).

2.5. Ion/metal analyses and translocation factor

Root and shoot material were digested by the method of Otte et al. (1993) with slight modification. Dried samples were cut into small pieces and then powdered in a grinding mill (Retsch MM 400, Germany) before acid digestion. Plant samples (0.2 g) were digested in 7 ml of HNO_3 in a Teflon reactor, Model KJ-180 (Preekem, China). Digested material was filtered using whatmann # 42 filter paper and $D.H_2O$ was finally added to obtain 10 ml of the total volume for each sample (Duarte et al., 2012). Sample dilutions were done as per requirement of standard elemental analysis. Shoot and root Pb was analyzed by flame atomic absorption spectrometry (Perkin Elmer Analyst 700 USA). Precision of the quality assurance was maintained by assessing the recovery of the analysis of matrix spike, its spike replicates and reagent blank as control for each of the batch samples. The percentage recovery of metals and metal spike were noted by the same procedure as described in Mujeeb et al. (2021). Cation analysis (Na^+ and K^+) was done by using flame photometer (Digital Clinical Flame photometer, model number I-65, Intech). Translocation factor (TF) of Pb was calculated according to the method of Marchiol et al. (2004) i.e., shoot to root Pb ratio.

2.6. Photosynthetic pigments

Fresh shoot material was freeze dried in a lyophilizer (SCANVAC, Coolsafe Touch, Denmark) for 4 h or till constant dry weights were obtained and then crushed in liquid nitrogen using mortar and pestle. Acetone (80 % v/v) was added to 0.1 g powdered material to make the total volume of 2 ml in Eppendorf tubes. The mixture was vortexed after which samples were kept in a refrigerator for 24 h. Photosynthetic pigments (chlorophylls 'a' and 'b' as well as carotenoids) were determined spectrophotometrically by the method of Lichtenthaler and Buschmann (2001). Chlorophyll fluorescence parameters i.e., Intrinsic quantum yield of photochemistry (Fv/Fm) and effective quantum yield of photosystem II (ϕ PSII) were determined on dark adapted shoots (30 min) with the help of pulse modulated fluorescence instrument (PAM 2500, Walz, Germany) (Shi et al., 2020).

2.7. Electrolyte leakage and hydrogen peroxide

Electrolyte leakage was determined on freshly cut shoots (0.2 gm) per replicate for each treatment after careful washing with distilled water. Shoot fresh weights were immediately noted. Shoot samples were inserted in screw capped test tubes containing 10 ml distilled water and initial electrical conductivity (EC_1) was noted after an hour with the help of CM-115 m, Kyoto Electronics, Japan). Samples were then autoclaved at 121 °C for 20 – 25 min, cooled to room temperature (25 °C) and then final conductivity (EC_2) was noted. Electrolyte leakage was calculated by:

$$\%\text{Electrolyte leakage} = EC_1/EC_2 * 100$$

For H_2O_2, fresh shoot tissues were mashed in liquid nitrogen and homogenized in trichloroacetic acid (1.8 ml of TCA, 3 % w/v). The extract was centrifuged (12,000 × *g*) for 20 min at 4 °C and the supernatant was used to determine H_2O_2 by spectrophotometric method (Loreto and Velikova, 2001).

2.8. Antioxidant enzymes

Freshly harvested shoot (0.1 g) samples from each treatment were crushed in a mortar and pestle using liquid nitrogen. The material was then homogenized in KH_2PO_4 buffer (pH 7.0) by adding 2 % PVPP (polyvinyl polypyrrolidone), 1 mM ascorbic acid and 5 mM EDTA. Homogenates were centrifuged at 12,000 × *g* for 20 min at 4 °C. Supernatant was used as aliquot for enzyme extract on which superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), Guaiacol peroxidase (GPX; EC 1.11.1.7) and Ascorbate peroxidase (APX, EC 1.11.1.1) were analyzed according to Hameed et al. (2012).

3. Osmolytes

3.1. Glycine betaine

Glycine betaine was determined spectrophotometrically in hot water extracts by the method of Grieve and Grattan (1983). Reference standards of betaine (50–200 μg/ml) were prepared in 1 N H_2SO_4 and GB calculated by plotting standard curve.

3.2. Proline

Proline was determined by using ninhydrin method of Bates et al. (1973) on hot water extracts. Free proline was calculated from a standard curve using analytical grade proline spectrophotometrically.

3.3. Total soluble sugars (TSS)

Total soluble sugars in plant hot water extracts were determined by the method of Gerhardt et al. (1994) by using anthrone reagent.

3.4. Statistical analyses

All statistical analysis was done by using SPSS software (Ver. 16, 2007). A One-way ANOVA was used to find out the significant differences across salinity and Pb treatments by using post hoc tests (Bonferroni / Welch / Games-Howell tests) showing significance ($P < 0.05$) and two- way ANOVA to calculate the combined effect of salinity (NaCl) and Pb.

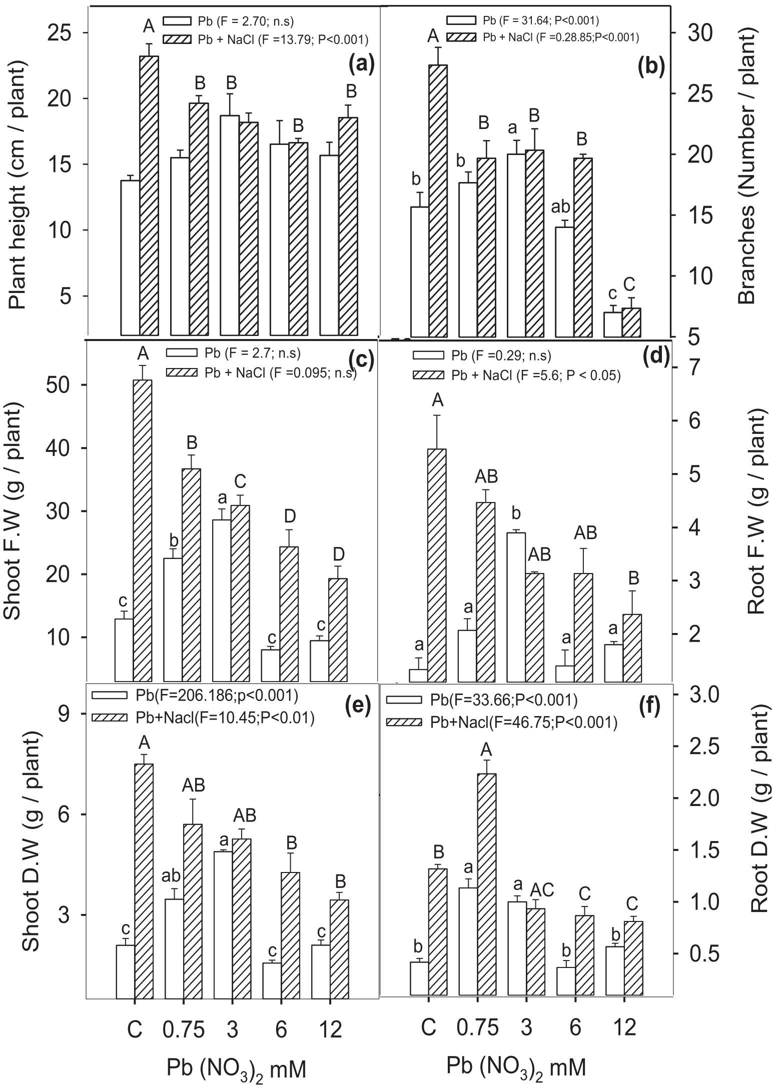

Fig. 1. Effects of different $Pb(NO_3)_2$ concentrations (0,0.75, 3, 6 and 12 mM) with NaCl (300 mM) and without NaCl (0 mM) on growth parameters: (**a**) Shoot FW, (**b**) Root FW, (**c**) Shoot DW, (**d**) Root DW, (**e**) Shoot length, (**f**) Root length, (**g**) number of branches and (**h**) Plant height of *Arthrocaulon machrostachyum*. Grouped error bars with different letters were significantly different at $p < 0.05$ (Bonferroni's test).

4. Results

4.1. Growth responses

Plant height, number of branches, shoot & root fresh weights (Fig. 1a –d) as well as their dry weights (Fig. 1e & f) optimized in saline (300 mM NaCl) treatments without Pb. However, in the absence of NaCl, plants showed a progressive increase in growth parameters up to 3 mM $Pb(NO_3)_2$ (Fig. 1c and d). Plant growth generally decreased with increasing Pb concentration under saline treatments (Fig. 1b–d). Reduced number of branches with low fresh and dry weights beyond 6 mM Pb indicated an overall poor growth response. Visible signs of injury with stunted growth appeared in 12 mM Pb by the end of 4th week with prominent shoot necrosis.

4.2. Water relations

Shoot water and osmotic potentials in *A. macrostachyum* decreased progressively (more negative) with the increases in Pb without NaCl (Fig. 2a and b). On the contrary, both shoot water and osmotic potential were similar up to 6 mM Pb (ranging between - 4.3 and −4.5 MPa) with NaCl but decreased substantially at 12 mM Pb (Fig. 2a and b). Similarly, shoot turgor was maintained up to 6 mM Pb (> 0.6 MPa) but significantly decreased in 12 mM both with and without NaCl (Fig. 2).

4.3. Lead accumulation in roots and shoots

Concentration of Pb in *A. macrostachyum* increased significantly ($P < 0.05$) under increasing $Pb(NO_3)_2$ with highest values in 12 mM i.e., 280–370 mg kg^{-1} in roots and 180–200 mg kg^{-1} in shoots (Fig. 2c & d). Translocation (TF) to shoot was > 1 up to 3 mM (1.53 in 0.75 mM and 2.16 in 0.75 mM + NaCl) while in 6 mM and higher Pb treatments, Pb allocation to roots was increased with lower TF values (0.56 in 12 mM Pb and 0.39 in 12 mM + NaCl).

4.4. Plant pigments and chlorophyll fluorescence

Plant pigments differed significantly ($P < 0.05$) among different Pb and NaCl treatments (Fig. 3a–d). Chlorophyll a, b and total chl were higher in control and NaCl treated plants alone (Fig. 3a–c). Carotenoid content remained consistently higher in all treatments except for 3 mM Pb with comparatively lower values (Fig. 3d). All plants treated with 6 mM or higher Pb either

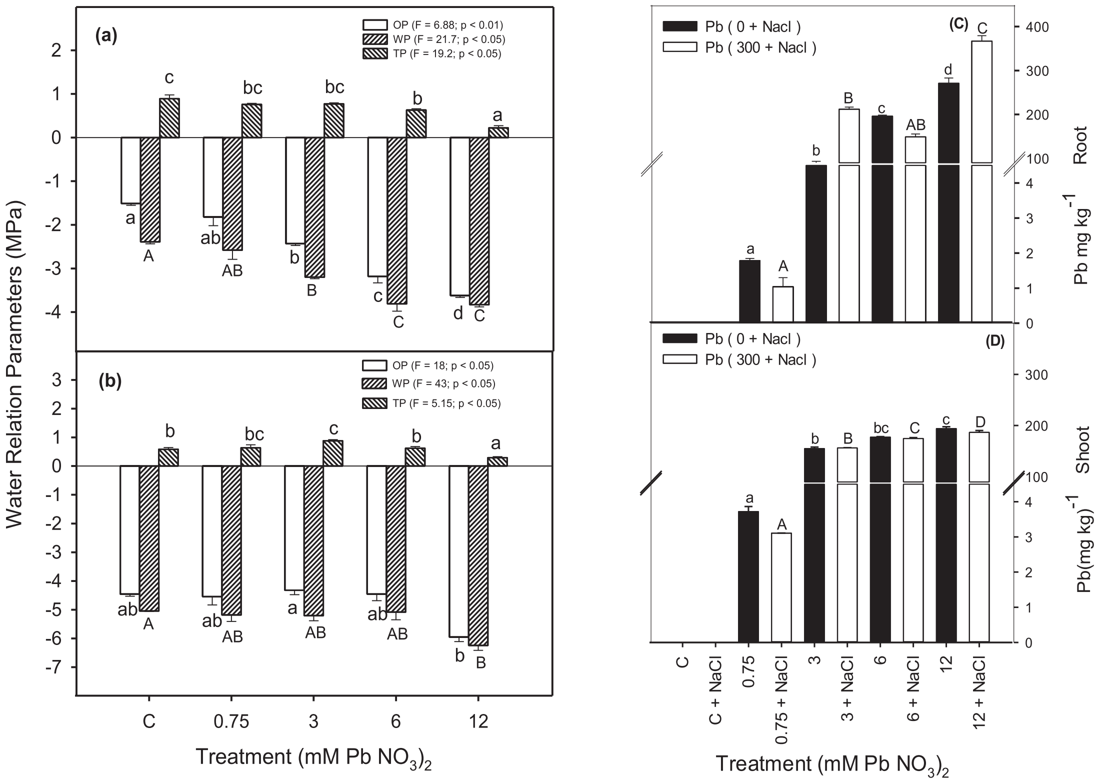

Fig. 2. Effects of different $Pb(NO_3)_2$ concentrations (0,0.75, 3, 6 and 12 mM) on shoot water relation parameters (left panel): **a**) without NaCl and **b**) with NaCl (300 mM). Inverted bars (negative values) represent water and osmotic potential and positive bars turgor potential (TP). Right panel shows Pb concentrations in roots (c) and shoots (d) of *Arthrocaulon machrostachyum*. Grouped error bars with different letters were significantly different at $p < 0.05$.

with or without NaCl had low chlorophyll content (Figs. 3a– c). The maximum quantum yield of foliar photochemistry (Fv/Fm) was 0.64 in 0 mM Pb, increased to 0.8 at 3 mM Pb; however, it was decreased in 6 and 12 mM Pb (0.6 and 0.54 respectively). Effective quantum yield (ϕ PSII) was 0.64 in 0 mM Pb both with and without NaCl with a transient increase in 3 mM + NaCl (0.74) and decreased to 0.64 and 0.58 in 6 mM and 12 mM Pb corresponding to decreases in chl 'a' and 'b'.

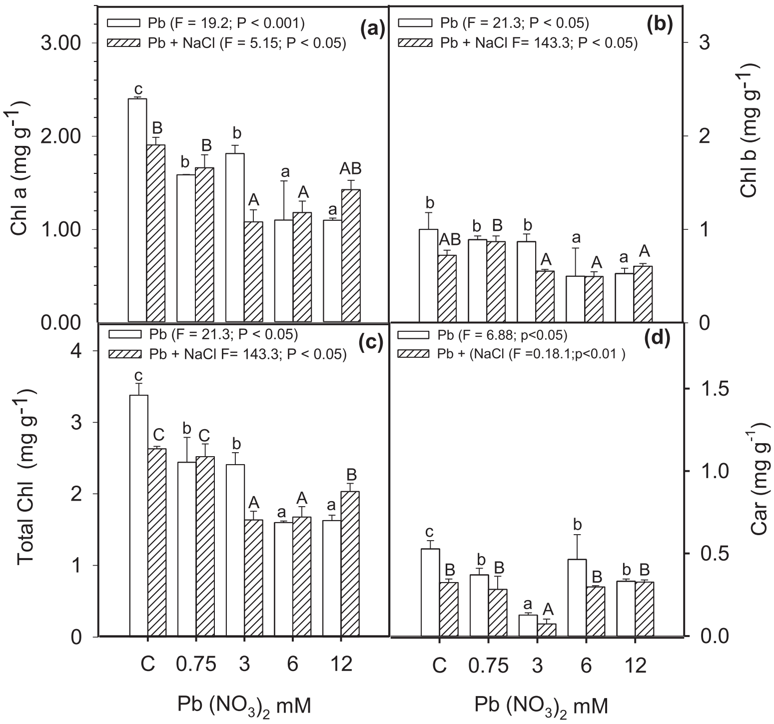

Fig. 3. Effects of different Pb $(NO_3)_2$ concentrations (0,0.75, 3, 6 and 12 mM) with 300 mM NaCl and without (0 mM) NaCl on shoot pigments: (**a**) Chlorophyll 'a', (**b**) Chlorophyll 'b', (**c**) total Chlorophyll and (**d**) Total carotenoids of *Arthrocaulon machrostachyum*. Grouped error bars with different letters were significantly different at $p < 0.05$ (Bonferroni's test).

Table 1
Two-way ANOVA for different growth, physiological and biochemical characteristics of *A. macrostachyum* grown under different Pb treatments with 300 mM NaCl and without (0 mM) NaCl salinity.

Parameters	Treatment	Salinity	Treatment * Salinity
Shoot Height	5.9^{**}	33.9^{***}	6.2^{**}
Root Height	5.0^{**}	8.2^{**}	5.6^{**}
Shoot FW	41.6^{***}	215.8^{***}	27.5^{***}
Root FW	6.7^{**}	46.4^{***}	12.8^{***}
Shoot DW	18.4^{***}	111.2^{***}	13.7^{***}
Root DW	64.8^{***}	128.4^{***}	20.2^{***}
Total Dry Biomass	40.8^{***}	217.2^{***}	29.8^{***}
Electrolyte Leakage	81.6^{***}	23.0^{***}	6.1^{**}
Pb Shoot	863.6^{***}	0.7 ns	0.5 ns
Pb Root	776.0^{***}	75.5^{***}	66.3^{***}
Na Shoot	13.1^{***}	1496.3^{***}	15.8^{***}
Na Root	9.0^{***}	90.8^{***}	9.3^{***}
K Shoot	33.9^{***}	12.5^{**}	4.6^{**}
K Root	30.7^{***}	0.3 ns	3.2^{*}
Catalase	17.6^{***}	0.01 ns	1.1 ns
APX	2.1 ns	3.0 ns	2.4 ns
SOD	1.8 ns	21^{**}	2.5^{*}
GPX	1.00 ns	15.8^{**}	0.6 ns
H_2O_2	35.6^{***}	22.2^{***}	4.5^{**}

Note: F values with asterisks (*) indicate significance level = * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.*s* = non-significant.

4.5. Cations (Na^+) and (K^+) content

A Two-Way ANOVA showed significant differences in root and shoot Sodium (Na^+) in NaCl treated plants (Table 1). Sodium concentration in shoot and root progressively increased with the increases in Pb in all NaCl treated plants compared to Control (Table 2). Highest root Na^+ was recorded in 12 mM with NaCl and lowest in 0.75 mM Pb without NaCl (Table 2). On the contrary, shoot Na^+ concentration was highest in 0.75 mM + NaCl treatment and significantly decreased with increases in Pb (Table 2). Root and shoot K^+ was unchanged up to 3 mM Pb both with and without NaCl however, a decreasing trend was observed with further increases in Pb concentrations (Table 2). Root Na/K ratio increased with increases in Pb + NaCl treatments (Table 2). A similar trend was observed for shoot Na/K ratio although values were comparatively lower than roots in plants treated with 3 mM or higher Pb (Table 2) further indicating low supply of Na^+ to shoot.

4.6. Organic osmolytes

Total soluble sugars (TSS) were significantly higher in shoots at 6 mM $Pb(NO_3)_2$ (Games-Howell test; Fig. 4a). However, in NaCl treated plants it was highest in 0.75 mM Pb (Fig. 4a). Proline (PRO) content linearly increased with increases in $Pb(NO_3)_2$ with and without NaCl (Fig. 4b). Shoot PRO was relatively higher in Pb treatments alone with highest values in 12 mM (Fig. 4b). A one-way ANOVA showed significant changes in TSS ($P < 0.01$) and PRO ($P < 0.05$) of *A. macrostachyum* under Pb treatments both with and without NaCl (Supplementary Table 1). Shoot glycinebetaine (GB) was ~ 4-fold higher than PRO in all treatments with highest values in 0.75 mM Pb + NaCl treatments (Fig. 4c). Significantly reduced shoot GB was observed in 12 mM Pb with and without NaCl (Fig. 4c).

4.7. Contribution of solutes to osmolality

Among organic osmolytes the contribution of GB to osmolality was highest ranging between 0.13 and 2.1 % (0.75 mM+ NaCl), followed by PRO (0.06 in C to 0.63 % in 12 mM Pb) and TSS (0.06 in C to 0.17 % in 0.75 mM Pb). However, among all tested solutes the contribution of Na+ was highest with maximum values (22 %) in 0.75 mM+ NaCl. Similarly, the contribution of *K*+ was also highest in 0.75 mM+ NaCl treatments with maximum values of 2.9 %.

4.8. Electrolyte leakage and hydrogen peroxide activities (H_2O_2)

Plants treated with 0.75 and 3 mM Pb both with and without NaCl showed low % electrolyte leakage while a significant increase was observed in 12 mM Pb with and without NaCl (Fig. 5a). The H_2O_2 content was transiently decreased in 3 mM Pb though its values were comparatively higher in 0.75 mM while significant increases were observed in 6 mM or higher Pb concentrations (Fig. 5b).

4.9. Antioxidant enzymes

The SOD activity increased with the addition of Pb and remained similar up to 6 mM $Pb(NO_3)_2$ without NaCl, however, its activity significantly increased with the increases in Pb under NaCl and decreased at 12 mM (Fig. 6a). The activity of CAT transiently decreased in 0.75 mM and 3 mM Pb both with and without NaCl while a significant increase was observed in 6 mM and 12 mM Pb (Fig. 6b). No change in the activity of GPx was found in all Pb treatments without NaCl but GPx was significantly increased in 6 and 12 mM Pb with NaCl (Fig. 6c). The activity of APx was marginally higher in 6 mM Pb without NaCl although no significant change was observed in any of the Pb treatments (Fig. 6d).

5. Discussion

Arthrocaulon macrostachyum optimized its growth in the presence of NaCl (300 mM) as commonly observed in obligate halophytes (Redondo-Gomez et al., 2010; Flowers and Colmer, 2008; Nisar et al., 2021) which may be related to improved water use efficiency and better nutrient acquisition (Ehsen et al., 2019). An improvement in

Table 2
Effects of different concentrations of $Pb(NO_3)_2$ (0, 0.75, 3, 6 and 12 mM) with 300 mM and without (0 mM) NaCl on Na^+ and K^+ concentration in roots and shoots of *Arthrocaulon macrostachyum*. Mean ± SE values with different letters (small letters without and capital letters with NaCl) are significantly different at $p < 0.05$ (Bonferroni's test).

Treatments	Root Na	Shoot Na	Root K	Shoot K	Root Na/ K ratio	Shoot Na / K ratio
$mmolkg^{-1}$ DW						
0	68.1 ± 6.2^{ab}	153.3 ± 7.5^{ab}	63.5 ± 8.2^{d}	141 ± 8.1^{d}	1.1 ± 0.14^{b}	1.09 ± 0.1^{a}
0+NaCl	580 ± 19.2^{B}	1663 ± 37.7^{AB}	76.75 ± 1.3^{D}	107 ± 1.1^{C}	7.55 ± 0.2^{A}	15.4 ± 0.51^{A}
250	25.4 ± 3.1^{a}	112 ± 5.8^{a}	66.9 ± 5.0^{d}	141 ± 11^{d}	0.38 ± 0.04^{a}	0.8 ± 0.02^{a}
250+NaCl	283 ± 12.6^{A}	2631 ± 211^{C}	21.32 ± 1.1^{A}	107 ± 2.21^{C}	13.3 ± 0.79^{B}	24.4 ± 1.9^{B}
1000	94.2 ± 2.2^{ab}	191.3 ± 11^{b}	53.30 ± 2.1^{c}	96.4 ± 3.33^{c}	1.77 ± 0.1^{b}	1.98 ± 18.3^{b}
1000+NaCl	1304 ± 63^{C}	1728 ± 33.7^{B}	53.94 ± 2.8^{C}	93.8 ± 3.75^{BC}	24.2 ± 0.6^{C}	18.4 ± 0.5^{A}
2000	65.2 ± 18.9^{ab}	140.3 ± 6.4^{ab}	31.98 ± 9.8^{b}	67.5 ± 16.9^{b}	2.05 ± 0.25^{bc}	2.11 ± 0.21^{c}
2000+NaCl	1739 ± 38^{CD}	1715.4 ± 84^{B}	38.80 ± 4.7^{B}	63.78 ± 3.75^{B}	44.32 ± 8.0^{D}	27.0 ± 1.9^{C}
4000	31.2 ± 2.0^{a}	121.2 ± 6.4^{a}	17.05 ± 2.3^{a}	17.63 ± 1.3^{a}	1.89 ± 0.21^{b}	6.89 ± 0.2^{d}
4000+NaCl	3029 ± 72^{D}	1447.5 ± 84^{A}	20.04 ± 3.0^{A}	37.52 ± 9.9^{A}	4.8 ± 2.3^{A}	42.6 ± 8.7^{D}

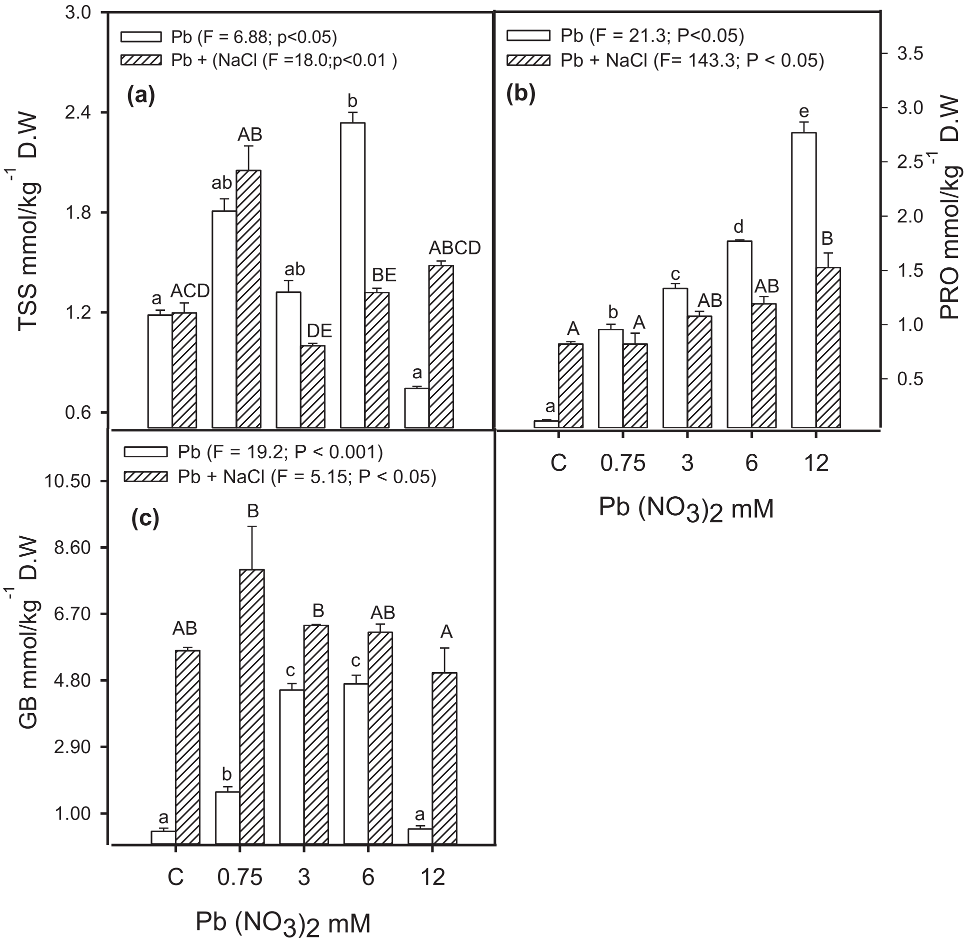

Fig. 4. Effects of different $Pb(NO_3)_2$ concentrations (0, 0.75, 3, 6 & 12 mM) with 300 mM NaCl and without (0 mM) NaCl on different organic osmolytes in shoots of *Arthrocaulon macrostachyum*: (**a**) Soluble sugars (TSS), (**b**) Proline (PRO) and (**c**) Glycine betaine (GB) in (mmol kg^{-1} DW). Grouped error bars with different letters were significantly different at $p < 0.05$ (Games-Howell test).

growth up to 3 mM $Pb(NO_3)_2$ in the absence of NaCl may be related to better nitrate acquisition as an osmolyte for maintaining turgor (Marschner, 2011) and not due to Pb alone; however, this assumption needs experimental testing in future studies. Conversely, the lower growth parameters in higher concentrations of $Pb(NO_3)_2$ (> 6 mM) were attributed to decreases in turgor potential (Koyro et al., 2013). Hence, the biological threshold of attaining cross tolerance of Pb and NaCl in *A. macrostachyum* was 6 mM but at the expense of growth. Progressive decreases in water and osmotic potentials in *A. macrostachyum* highlighted a typical osmo-conformor response in this species (Khan et al., 2005). The phenomenon of reduced turgor in 6 mM or higher Pb concentrations appeared to be a consequence of cell wall thickening (Rucińska-Sobkowiak et al., 2016; Krzesłowska, 2011) and reduced cell elongation (Pawlak-Sprada et al., 2011).

Photosynthetic pigments play a crucial role in energy metabolism under stressed conditions (Patel and Parida, 2021; Ma et al. 2021; He et al., 2023). Higher levels of foliar pigments were observed in plants growing in NaCl, but substantial decreases with increasing Pb $(NO_3)_2$ + NaCl revealed the combined and synergistic effects of metal and ionic stress. The reduction of photosynthetic pigments in 6 mM and higher Pb treatments may be linked with enzyme instability along with degradation of protein complexes of photosynthetic pigments (Patel and Parida, 2021) as their biosynthesis is often disrupted with increasing metal stress (Helaoui et al., 2023). Carotenoids, as non-antioxidants are also effective in protecting plant photosynthetic machinery under stress (Ghanem et al., 2021) and consistently higher CAR in *A. macrostachyum* (except for 3 mM Pb) seemed effective strategy. Similarly, decreases in foliar chlorophyll fluorescence i.e., *Fv*/*Fm* (potential photo-chemical quantum yield) and ϕ PSII (effective photochemical efficiency) in 6 and 12 mM Pb concentrations may be linked to disrupted photosystems and lower net carbon assimilation of *A. macrostachyum* as observed in other species (Ehsen et al., 2019; Ma et al., 2021; Wu et al., 2023).

5.1. Sodium (Na^+), potassium (K^+) and lead (Pb) content

The plants managed to have normal shoot growth while maintaining Na^+ content of up to 3 mM Pb with transiently decreasing root Na^+; however, root Na^+ substantially increased in Pb concentrations higher than 6 mM compared to shoot indicating lower translocation. In this experiment, a two-fold increase in root Pb levels with highest Na^+ in 12 mM $Pb(NO_3)_2$ was indicative of possible hampered Pb transport to shoots via possible blockage of channels (Pourrut et al., 2011). The accumulation of ~ 200 mg kg^{-1} Pb in shoots indicated that the *A. macrostachyum* plants have some phytoremediation competence (Aziz and Mujeeb, 2022). To be designated as a typical hyperaccumulating species for specific heavy metals or metalloids, the plants should accumulate over 1000 mg kg^{-1} of Pb (Van der Ent et al., 2013). Root and shoot K^+ consistently decreased with the increases in Pb and so as Na/K ratio. Potassium (K^+) is known to maintain plant growth and its lower values may be directly related to growth reduction (Munns et al., 2019; de Bang et al., 2021). Salt tolerant plants often use Na^+ as cost effective osmoticum in place of K^+ (Munns et al., 2019; He et al., 2023; Wu et al., 2023) as observed in this study.

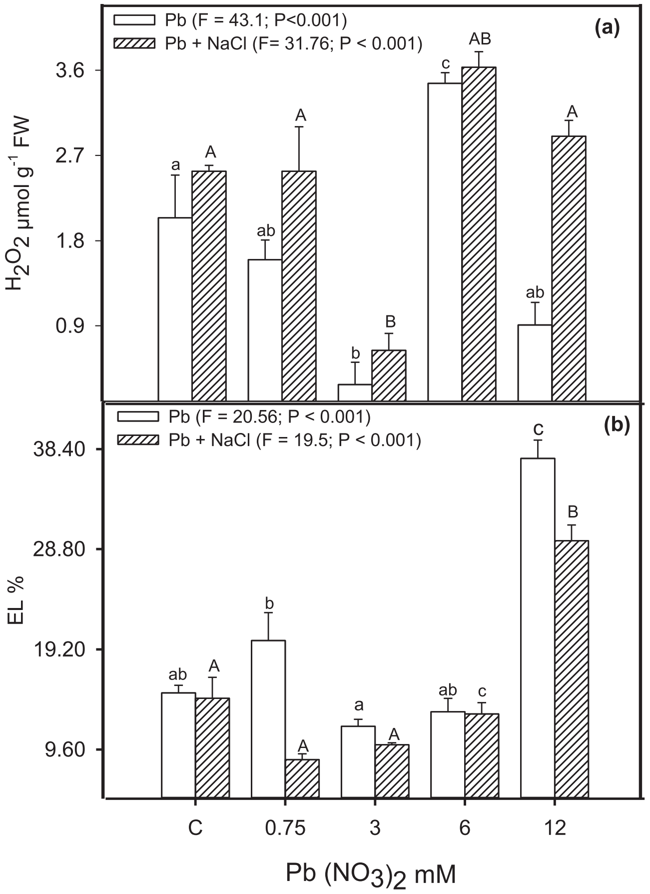

Fig. 5. Effects of different Pb $(NO_3)_2$ concentrations (0, 0.75, 3, 6 and 12 mM) with 300 mM NaCl and without (0 mM) NaCl on shoot damage markers: (**a**) Hydrogen peroxide (H_2O_2) in μmol g^{-1} FW, (**b**) Electrolyte leakage (EL%) in *Arthrocaulon machrostachyum*. Grouped error bars with different letters were significantly different at $p < 0.05$ (Bonferroni's test).

Halophytes are reported to exclude salts via trichomes or salt glands and those lacking this mechanism tend to increase shoot succulence (Flowers et al., 2008). Higher shoot succulence in *A. macrostachyum* could have assisted in achieving optimized water balance (Katschnig et al., 2013; Patel and Parida, 2021) and avoiding phytotoxicity attributed to elevated Pb and NaCl levels as in other marsh plants (Wang et al., 2012).

5.2. Organic osmolytes

Osmolytes are low molecular weight, readily soluble and non-toxic substances that helps in cellular osmotic adjustment without disrupting metabolism (Slama et al., 2015). Such compounds can also serve as osmoprotectants for sub cellular structures by preventing them from oxidative damage (Bose et al., 2014, 2015). Unlike glycophytes, salt resistant plants could use Na^+ in place of K^+ for osmotic adjustment (Hameed et al., 2021; He et al., 2023). A combination of both organic and inorganic solutes seems to achieve osmotic balance by maintaining cell turgor (Hameed et al., 2021). Total soluble sugars (TSS) not only play a major role in metabolic processes (e.g., respiration, photosynthesis etc.) but also assist in coping with ROS generation (Annunziata et al., 2019). In this study, TSS was highest in 6 mM Pb, although its contribution to osmolality was lower compared to PRO and GB. Among organic solutes, GB had highest values as well as percent contribution followed by PRO in *A. macrostachyum* however, Na^+ appeared to contribute the most in osmolality for its higher content (Flowers and Colmer, 2008; Wu et al., 2023). Plants usually accumulate some trace metals in different organs although their higher values could be phytotoxic that may increase cytosolic acidity (Amjad et al., 2022; Gupta et al., 2009), damage photosynthetic pigments and enhances ROS generation leading to apoptosis and cell death (Natasha et al., 2019; Rafiq et al., 2018; Zhao et al., 2018; Shahid et al., 2017). Proline (PRO) and TSS appeared to function as osmoprotectants in *A. macrostachyum* under stress as found in other halophytes (Hameed et al., 2021; Annunziata et al., 2019). Moreover, PRO can also help in chelation of metals that helps in reducing their toxicity (Wiszniewska et al., 2019). Linear increases in PRO content of *A. macrostachyum* with the increases in Pb and NaCl could be related to ROS scavenging as has been reported in some studies on drought and salt tolerance (Thakur and Sharma, 2016; Wiszniewska et al., 2019; Lamhamdi et al., 2013; Hameed et al., 2021). Glycine betaine (GB) is a quaternary ammonium compound often found in chenopods (Chen and Murata, 2011) and meant for the protection of PS-II machinery (Ashraf et al., 2019). Higher GB content in *A. machrostachyum* may be related to the protection of D1 protein (Chen and Murata, 2011). However, a significant decrease in GB for test plants growing in 12 mM Pb (with and without salinity) indicated that *A. machrostachyum* plants were unable to manage oxidative stress which was also manifested in visible signs of injury. The concomitant decreases in foliar fluorescence values (Fv/Fm and ϕ PSII) further supported this observation pertaining to the disruption of photosynthetic apparatus (Ma et al., 2021).

5.3. Stress markers and antioxidant enzyme activities

Plants surviving in stressed environments generates various types of ROS when exposed to metal ions, but they are detoxified in the presence of enzymatic and non-enzymatic antioxidants (Amjad et al., 2022; Hasanuzzaman et al., 2020). Levels of H_2O_2 and electrolyte leakage (%EL) usually represents ROS generation and damage to the membrane systems (Hameed et al., 2012). In this study, transient decreases of EL and H_2O_2 up to 3 mM treatments complemented with better growth and physiological parameters, while significantly higher values in 12 mM point towards membrane disruption.

Superoxide dismutase (SOD) primarily act as a defensive tool for protecting plants against oxidative damage as it converts superoxide radicals ($\dot{O}^-$) into relatively less toxic compound i.e., H_2O_2 (Iftikhar et al., 2021; Natasha et al., 2019). However, other antioxidative enzymes helps in further detoxification of H_2O_2 by breaking down into water and oxygen (Iftekhar et al., 2021; Rehman et al., 2019; Shahid et al., 2017). Consistent increases in SOD up to 6 mM Pb in *A. macrostachyum* epitomizes steady ROS scavenging though, a sudden decrease in 12 mM Pb both with and without salinity suggests failure of SOD in ROS detoxification (Rehman et al., 2019). Other enzymes for example, CAT, GR and peroxidases are also involved in ROS detoxification (Patel and Parida, 2021). Among them, increased levels of GPX indicates both ROS quenching as well as cell wall hardening (Yildiztugay et al., 2014) as observed in shoots of water hyacinth in response to Pb stress (Malar et al., 2014). The reason could be impaired redox homeostasis following ROS generation (Maqsood et al., 2023). The overproduction of ROS is cytotoxic which can damage chloroplast membrane system, enzyme activity, protein, and lipo-proteins (Gill and Tuteja, 2010). Earlier research has demonstrated that the various enzyme activities may be hampered due to metal toxicity (Anjitha et al., 2021). In the current study, there was no significant change in GPx up to 3 mM Pb; however, increased activity in 6 and 12 mM Pb coupled with NaCl was observed in *A. macrostachyum*. The reason of increased GPx activity may have either caused cell wall hardening (Yildiztugay et al., 2014) or having lower indole acetic acid (auxin) in the test species resulting in growth reduction (Hameed et al., 2012). The higher GPx with reduced growth in *A. macrostachyum* supported the above-mentioned assumptions.

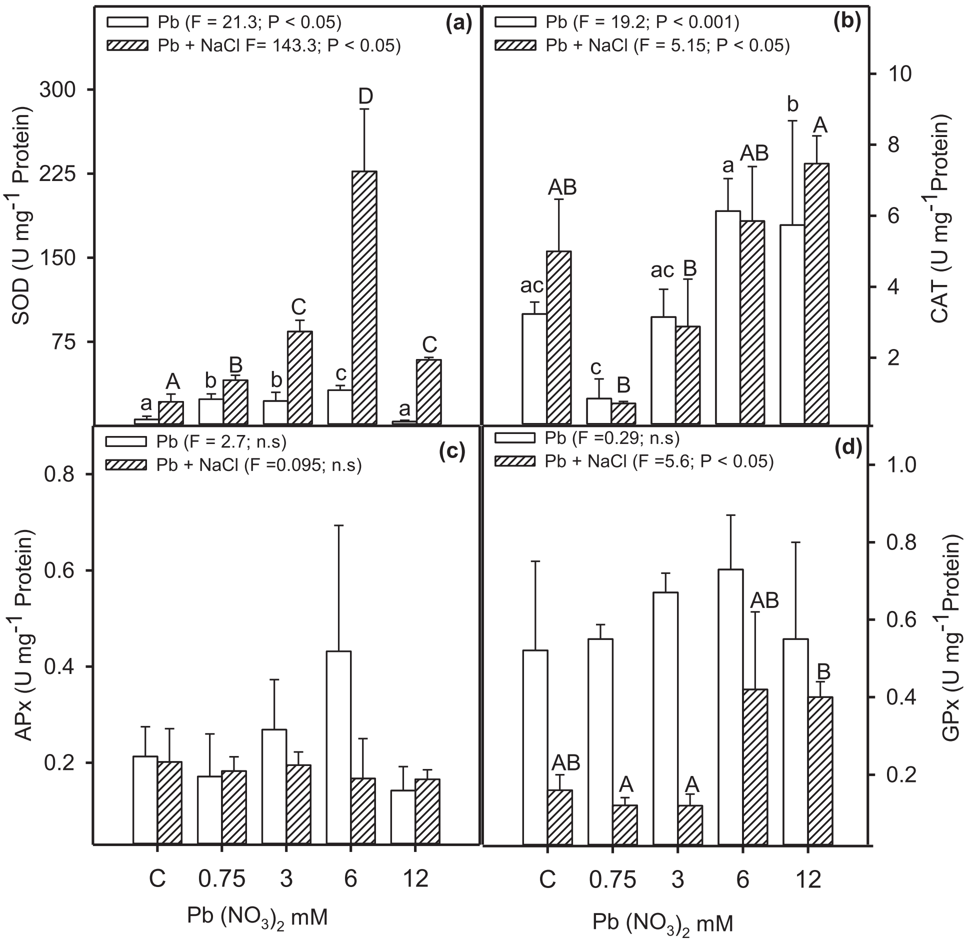

Fig. 6. Effects of different Pb $(NO_3)_2$ concentrations (0, 0.75, 3, 6 and 12 mM) with 300 mM NaCl and without (0 mM) NaCl on the activity of antioxidant enzymes in shoots: (**a**) Superoxide dismutase (SOD), (**b**) Catalase (CAT), (c) Ascorbate peroxidase (APX) and (d) Guiacol peroxidase (GPX) expressed in U mg $^{-1}$ protein of *Arthrocaulon machrostachyum*. Grouped error bars with different letters were significantly different at $p < 0.05$ (Games-Howell test).

A transient decrease in CAT activity at 0.75 and 3 mM Pb could also be related to low H_2O_2 while a noteworthy increase in 6 mM Pb suggest plant's ability to tolerate higher H_2O_2 production (Amjad et al., 2022) and activation of stress-responsive antioxidative metabolism under saline conditions (Rehman et al., 2019). The activity of APx enzyme was marginally increased in 6 mM Pb without NaCl although no change was observed in any of the Pb treated plants. Marginal increases in APx at 6 mM could be related to increases in TSS which reflects AsA biosynthesis, a substrate for APx enzyme (Nisar et al., 2021). This enzyme is known to be involved in detoxification of ROS at chloroplast level where CAT activity is usually low or absent (Rahman et al., 2021). However, to confirm whether ROS production at Pb concentrations of 6 and 12 mM was chloroplastic or cytoplasmic needs further testing on different isozymes in *A. macrostachyum* (Nisar et al., 2021). These findings suggested that a well coordinated antioxidant system helped in the ROS scavenging process and up to 3 mM; while the plants were unable to cope with 12 mM Pb possibly due to chloroplast membrane damage.

The following conclusions could be inferred from the current research:

1. Concentration of Pb in *Arthrocaulon macrostachyum* at 6 mM or higher treatments increased Pb allocation to roots compared to shoots indicating limited vascular translocation.
2. Dual tolerance to salinity and Pb in *A. macrostachyum* was achieved by antioxidant enzyme feedback of up to 3 mM Pb $(NO_3)_2$, efficient ROS scavenging, enhanced plant pigments and tissue turgor. However, the 12 mM Pb treated plants grew poorly, developed visible injury symptoms and with lower chlorophyll levels.
3. Due to its high Pb accumulation ability under saline conditions, *Arthrocaulon macrostachyum* may be considered as a suitable species for phytoremediation of saline polluted lands.

6. Conclusions

The wetland halophyte *Arthrocaulon macrostachyum* can tolerate Pb concentrations of up to 6 mM while naturally thriving in coastal salt marshes. Despite having lower growth beyond 3 mM $Pb(NO_3)_2$, the *A. macrostachyum* plants were able to manage salinity and Pb dual tolerance possibly through optimised antioxidant feedback. This stem succulent halophyte showed Pb accumulation of up to 3 mM; but in concentrations higher than 6 mM Pb, the accumulation was more pronounced in roots. Nevertheless, the test species may be grown for Pb phytoextraction from polluted areas. This study offered an innovative way to harness the potential of wetland halophytes for phytoremediation of polluted lands. Moving forward, the molecular mechanisms underlying the unique physiological performances of halophytes and elevated Pb levels require further studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Azhar Manzoor: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Irfan Aziz:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Amtul Mujeeb:** Formal analysis, Data curation. **Zainul Abideen:** Writing – review & editing, Validation. **Jean Wan Hong Yong:** Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2024.10.006.

References

Alamgir, A., Ali, Q., Fatima, N., Khan, M., Nawaz, M.F., Tariq, S., Rizwan, M., Yong, J.W.H, 2024. Geospatial quality assessment of locally available ice for heavy metals and metalloids and their potential risks for human health in Karachi, Pakistan. Heliyon 10, e28252. https://doi.org/10.1016/j.heliyon.2024.e28252.

Amari, T., Ghnaya, T., Abdelly, C., 2017. Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. S. Afr. J. Bot. 111, 99–110. https://doi.org/10.1016/j.sajb.2017.03.011.

Amjad, M., Iqbal, M.M., Abbas, G., Farooq, A.B.U., Naeem, M.A., Imran, M., Jacobsen, S.E., 2022. Assessment of cadmium and lead tolerance potential of quinoa (*Chenopodium quinoa* Willd) and its implications for phytoremediation and human health. Environ. Geochem. Health 44 (5), 1487–1500. https://doi.org/10.1007/s10653-021-00826-0(0123456789.

Anjitha, K.S., Sameena, P.P., Puthur, J.T., 2021. Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. Plant Stress 2, 100038. https://doi.org/10.1016/j.stress.2021.100038.

Annunziata, M.G., Ciarmiello, L.F., Woodrow, P., Dell'Aversana, E., Carillo, P., 2019. Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. Front. Plant Sci. 10, 230. https://doi.org/10.3389/fpls.2019.00230.

Ashraf, S., Ali, Q., Zahir, Z.A., Ashraf, S., Asghar, H.N., 2019. Phytoremediation: environmentally sustainable way for reclamation of heavy metal polluted soils. Ecotoxicol. Environ. Saf. 174, 714–727. https://doi.org/10.1016/j.ecoenv.2019.02.068.

Aziz, I., Mujeeb, A., 2022. Halophytes for phytoremediation of hazardous metal(loid)s: a terse review on metal tolerance, bio-indication and hyperaccumulation. J. Hazard Mater. 424, 127309. https://doi.org/10.1016/j.jhazmat.2021.127309.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39 (1), 205–207.

Bernard, A., 2008. Cadmium and its adverse effects on human health. Indian. J. Med. Res. 128 (4), 557.

Bose, J., Rodrigo-Moreno, A., Lai, D., Xie, Y., Shen, W., Shabala, S., 2015. Rapid regulation of the plasma membrane H+-ATPase activity is essential to salinity tolerance in two halophyte species, *Atriplex lentiformis* and *Chenopodium quinoa*. Ann. Bot. 115 (3), 481–494. https://doi.org/10.1093/aob/mcu219.

Bose, J., Rodrigo-Moreno, A., Shabala, S., 2014. ROS homeostasis in halophytes in the context of salinity stress tolerance. J. Exp. Bot. 65 (5), 1241–1257. https://doi.org/10.1093/jxb/ert430.

Chen, T., Murata, N., 2011. Glycine betaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant Cell Environ. 34, 1–20. https://doi.org/10.1016/j.tplants.2008.06.007.

Christofilopoulos, S., Syranidou, E., Gkavrou, G., Manousaki, E., Kalogerakis, N., 2016. The role of halophyte *Juncus acutus L.* in the remediation of mixed contamination in a hydroponic greenhouse experiment. J. Chem. Technol. 91 (6), 1665–1716. https://doi.org/10.1002/jctb.4939.74.

de Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New Phytol. 229, 2446–2469. https://doi.org/10.1111/nph.17074.

Duarte, B., Silva, V., Cacador, I., 2012. Hexavalent chromium reduction, uptake and oxidative biomarkers in *Halimione portulacoides*. Ecotoxicol. Environ. Saf. 83, 1–7. https://doi.org/10.1016/j.ecoenv.2012.04.026.

Ehsen, S., Abideen, Z., Rizvi, R.F., Gulzar, S., Aziz, I., Gul, B., Ansari, R., 2019. Ecophysiological adaptations and anti-nutritive status of sustainable cattle feed *Haloxylon stocksii* under saline conditions. Flora 257, 151425. https://doi.org/10.1016/j.flora.2019.151425.

Ekmekci, Y., Tanyolaç, D., Ayhan, B., 2009. A crop tolerating oxidative stress induced by excess lead: maize. Acta Physiol. Plant. (2), 319–330. https://doi.org/10.1007/s11738-008-0238-3.

Epstein, E., 1972. Mineral Nutrition of plants: Principles and Perspectives. J. Wiley and Sons, Inc., New York, pp. 68–82.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963. https://www.jstor.org/stable/25150520.

Gairola, S., Bhatt, A., El-Keblawy, A., 2015. A perspective on potential use of halophytes for reclamation of salt-affected lands. Wulfenia J. 22, 88–97.

Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R., 1994. Methods for general and molecular microbiology. Am. Soc. Microbiol. Washington, D.C.

Ghanem, A.F.M., Mohamed, E., Kasem, A.M.M.A., El-Ghamery, A.A., 2021. Differential salt tolerance strategies in three halophytes from the same ecological habitat: augmentation of antioxidant enzymes and compounds. Plants 10, 1100. https://doi.org/10.3390/plants10061100.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48 (12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016.

Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70 (2), 303–307.

Gupta, D.K., Nicoloso, F.T., Schetinger, M.R.C., Rossato, L.V., Pereira, L.B., Castro, G.Y., Tripathi, R.D., 2009. Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. J. Hazard. Mater. 172 (1), 479–484. https://doi.org/10.1016/j.jhazmat.2009.06.141.

Hameed, A., Ahmed, M.Z., Hussain, T., Aziz, I., Ahmad, N., Gul, B, Nielsen, B.L., 2021. Effects of salinity stress on chloroplast structure and function. Cells 10, 2023. https://doi.org/10.3390/cells10082023.

Hameed, A., Hussain, T., Gulzar, S., Aziz, I., Gul, B., Khan, M.A., 2012. Salt tolerance of a cash crop halophyte *Suaeda fruticosa*: biochemical responses to salt and exogenous chemical treatments. Acta Physiol. Plant. 34 (6), 2331–2340. https://doi.org/10.1007/s11738-012-1035-6.

Haghighizadeh, A., Rajabi, O., Nezarat, A., Hajyani, Z., Haghmohammadi, M., Hedayatikhah, S., Beni, A.A, 2024. Comprehensive analysis of heavy metal soil contamination in mining environments: impacts, monitoring Techniques, and remediation strategies. Arab. J. Chem., 105777. https://doi.org/10.1016/j.arabjc.2024.105777.

Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fujita, M., Fotopoulos, V., 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants 9, 681. https://doi.org/10.3390/antiox9080681.

He, A., Ma, Z., Li, Y., Huang, C., Yong, J.W.H., Huang, J., 2023. Spatiotemporal, physiological and transcriptomic dynamics of wild jujube seedlings under saline conditions. Tree Physiol. 43, 832–850. https://doi.org/10.1093/treephys/tpad001.

Helaoui, S., Boughattas, I., Mkhinini, M., Chebbi, L., Elkribi-Boukhris, S., Alphonse, V., Livet, A., Banni, M., Bousserrhine, N., 2023. Biochar amendment alleviates heavy metal phytotoxicity of *Medicago sativa* grown in polymetallic contaminated soil: evaluation of metal uptake, plant response and soil properties. Plant Stress 10, 100212. https://doi.org/10.1016/j.stress.2023.100212.

Iftekhar, A., Abbas, G., Saqib, M., Shabbir, A., Amjad, M., Shahid, M., Qaisrani, S.A., 2021. Salinity modulates lead (Pb) tolerance and phytoremediation potential of quinoa: a multivariate comparison of physiological and biochemical attributes. Environ. Geochem. Health 44 (1), 257–272. https://doi.org/10.1007/s10653-021-00937-8.

Jalil, S., Nazir, M.M., Ali, Q., Zulfiqar, F., Moosa, A., Altaf, M.A., Zaid, A., Nafees, M.A., Yong, J.W.H., Jin, X., 2023. Zinc and nano zinc mediated alleviation of heavy metals and metalloids in plants: an overview. Funct. Plant Biol. 50, 23021. https://doi.org/10.1071/FP23021.

Jiang, W., Liu, D., 2010. Pb-induced cellular defense system in the root meristematic cells of *Allium sativum* L. BMC Plant Biol. 10 (1), 1–8.

Jurado, C., Díaz-Vivancos, P., Gregorio, B.E., Acosta-Motos, J.R., Hernández, J.A., 2024. Effect of halophyte-based management in physiological and biochemical responses of tomato plants under moderately saline greenhouse conditions. Plant Physiol. Biochem. 206, 108228. https://doi.org/10.1016/j.plaphy.2023.108228.

Katschnig, D., Broekman, R., Rozema, J., 2013. Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: growth, morphology and physiology. Environ. Exp. Bot. 92, 32–42.

Khan, M.A., Ungar, I.A., Showalter, A.M., 2005. Salt stimulation and tolerance in an intertidal stem-succulent halophyte. J. Plant Nutr. 28, 1365–1374. https://doi.org/10.1081/PLN-200067462.

Khilji, S.A., Munir, N., Aziz, I., Anwar, B., Hasnain, M., Jakhar, M.A., Yang, H.H., 2022. Application of algal nanotechnology for leather wastewater treatment and heavy metal removal efficiency. Sustainability 14 (21), 13940.

Korzeniowska, J., Stanislawska-Glubiak, E., 2015. Phytoremediation potential of *Miscanthus giganteus* and *Spartina pectinata* in soil contaminated with heavy metals. Environ. Environ. Sci. Pollut. 22 (15), 11648–11657. https://doi.org/10.1007/s11356-015-4439-1.

Koyro, H.-W., Hussain, T., Huchzermeyer, B., Khan, M.A., 2013. Photosynthetic and growth responses of a perennial halophytic grass *Panicum turgidum* to increasing NaCl concentrations. Environ. Exp. Bot. 91, 22–29. https://doi.org/10.1016/j.envexpbot.2013.02.007.

Krzesłowska, M., 2011. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta Physiol. Plant. 33 (1), 35–51. https://doi.org/10.1007/s11738-010-0581-z.

Lamhamdi, M., El Galiou, O., Bakrim, A., Ńovoa-Mũnoz, J., Arias-Est́evez, M., Aarab, A., Lafont, R., 2013. Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. Saudi J. Biol. Sci. 20, 29–36 . https://doi.org/10.1016/j.sjbs.2012.09.001.

Li, M., Heng, Q., Wang, Z., Jiang, Y., Wang, X., He, X., Yong, J.W.H., Dawoud, T.M., Rahman, S.U., Fan, J., 2024. Phytoremediation efficiency of poplar hybrid varieties with diverse genetic backgrounds in soil contaminated by multiple toxic metals

(Cd, Hg, Pb, and As). Ecotoxicol. Environ. Saf. 283, 116843. https://doi.org/10.1016/j.ecoenv.2024.116843.

Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV vis spectroscopy. Curr. Protoc. Food Anal. 1 (1). https://doi.org/10.1002/0471142913.faf0403s01 F4-3.

Liu, L., Zhang, Q., Hu, L., Tang, J., Xu, L., Yang, X., Yong, J.W.H., Chen, X., 2012. Legumes can increase cadmium contamination in neighboring crops. Plos One 7, e42944. https://doi.org/10.1371/journal.pone.0042944.

Lokhande, V.H., Suprasanna, P., 2012. Prospects of Halophytes in Understanding and Managing Abiotic Stress tolerance. In Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. Springer, New York, NY, pp. 29–56. https://doi.org/10.1007/978-1-4614-0815-4_2.

Loreto, F., Velikova, V., 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 127 (4), 1781–1787. https://doi.org/10.1104/pp.010497.

Lutts, S., Lefèvre, I., 2015. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? Ann. Bot. 115 (3), 509–528. https://doi.org/10.1093/aob/mcu264.

Ma, M.Z., Liu, Y.F., Bai, C.M., Yong, J.W.H., 2021. The significance of chloroplast NAD (P) H dehydrogenase complex and its dependent cyclic electron transport in photosynthesis. Front. Plant Sci. 12, 1–8. https://doi.org/10.3389/fpls.2021.661863.

Malar, S., Vikram, S.S., Favas, P.J.C., Peruma, V., 2014. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Bot. Stud. 55, 54. http://www.as-botanicalstudies.com/content/55/1/54.

Manousaki, E., Galanaki, K., Papadimitriou, L., Kalogerakis, N., 2014. Metal phytoremediation by the halophyte *Limoniastrum monopetalum (L.)* Boiss: two contrasting ecotypes. Int. J. Phytorem. 16 (7−8), 755–769. https://doi.org/10.1080/15226514.2013.856847.

Marchiol, L., Assolari, S., Sacco, P., Zerbi, G., 2004. Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multi contaminated soil. Environ. Pollut. 132 (1), 21–27. https://doi.org/10.1016/j.envpol.2004.04.001.

Marschner, H., 2011. Marschner's Mineral Nutrition of Higher Plants. Academic Press, London.

Manousaki, E., Kalogerakis, N., 2011. Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. Ind Eng Chem Res 50, 656–660. https://doi.org/10.1021/ie100270x.

Maqsood, M.F., Shahbaz, M., Khalid, F., Rasheed, Y., Asif, K., Naz, N., Zulfiqar, U., Zulfiqar, F., Moosae, A., Alamer, K.H., Attia, H., 2023. Biogenic nanoparticles application in agriculture for ROS mitigation and abiotic stress tolerance: a review. Plant Stress 10, 100281. https://doi.org/10.1016/j.stress.2023.100281.

Martínez-Sánchez, M.J., García-Lorenzo, M.L., Pérez-Sirvent, C., Bech, J., 2012. Trace element accumulation in plants from an aridic area affected by mining activities. J. Geochem. Explor. 123, 8–12. https://doi.org/10.1016/j.gexplo.2012.01.007.

Mujeeb, A., Aziz, I., Ahmed, M.Z., Alvi, S.K., Shafiq, S., 2020. Comparative assessment of heavy metal accumulation and bio-indication in coastal dune halophytes. Ecotoxicol. Environ. Saf. 195, 110486. https://doi.org/10.1016/j.ecoenv.2020.110486.

Mujeeb, A., Aziz, I., Ahmed, M.Z., Shafiq, S., Fatima, S., Alvi, S.K., 2021. Spatial and seasonal metal variation, bioaccumulation & biomonitoring potential of halophytes from littoral zones of the Karachi Coast. Sci. Total Environ.. https://doi.org/10.1016/j.scitotenv.2021.146715.

Munns, R., Gilliham, M., 2015. Salinity tolerance of crops - what is the cost? New Phytol. 208, 668–673. https://doi.org/10.1111/nph.13519.

Munns, R., Day, D.A., Fricke, W., Watt, M., Arsova, B., Barkla, B.J., Bose, J., Byrt, C.S., Chen, Zhong-Hua, Foster, K.J., Gilliham, M., Henderson, S.W., Jenkins, C.L.D., Kronzucker, H.J., Miklavcic, S.J., Plett, D., Roy, S.J., Shabala, S., Shelden, M.C., Soole, K.L., Taylor, N.L., Tester, M., Wege, S., Wegner, L.H., Tyerman, S.D, 2019. Energy costs of salt tolerance in crop plants. New Phytol. 225, 1072–1090. https://doi.org/10.1111/nph.15864.

Natasha, M.S., Dunat, C., Khalid, S., Rabbani, F., Farooq, A.B.U., Amjad, M., Abbas, G., Niazi, N.K., 2019. Foliar uptake of arsenic nanoparticles by spinach: an assessment of physiological and human health risk implications. Environ. Sci. Pollut. Res. 26, 20121–20131. https://doi.org/10.1007/s11356-018-3867-0.

Nikalje, G.C., Suprasanna, P., 2018. Coping with metal toxicity−cues from halophytes. Front. Plant Sci. 9, 777. https://doi.org/10.3389/fpls.2018.00777.

Ningombam, L., Hazarika, B.N., Yumkhaibam, T., Heisnam, P., Singh, Y.D., 2024. Heavy metal priming plant stress tolerance deciphering through physiological, biochemical, molecular and omics mechanism. S. Afr. J. Bot. 168, 16–25. https://doi.org/10.1016/j.sajb.2024.02.032.

Nisar, F., Gul, B., Aziz, I., Hameed, A., Egan, T., 2021. Increasing salinity leads to differential growth and H_2O_2 homeostasis in plants produced from heteromorphic seeds of the succulent halophyte *Arthrocnumum indicum*. Plant Physiol. Biochem. 166, 225–234. https://doi.org/10.1016/j.plaphy.2021.05.029.

Otte, M.L., Haarsma, M.S., Broekman, R.A., Rozema, J., 1993. Relation between heavy metal concentrations in salt marsh plants and soil. Environ. Pollut. 82 (1), 13–22. https://doi.org/10.1016/0269-7491(93)90157-J.

Paraskevopoulou, A., Mitsios, I., Fragkakis, I., et al., 2015. The growth of *Arthrocnemum macrostachyum* and *Halimione portulacoides* in an extensive green roof system under two watering regimes. Agric. Sci. Proc. 4, 242–249.

Patel, M., Parida, A.K., 2021. Salinity alleviates the arsenic toxicity in the facultative halophyte *Salvadora persica* L. by the modulations of physiological, biochemical, and ROS scavenging attributes. J. Hazard. Mater. 401, 123368. https://doi.org/10.1016/j.jhazmat.2020.123368.

Pawlak-Sprada, S., Arasimowicz-Jelonek, M., Podgórska, M., Deckert, J., 2011. Activation of phenylpropanoid pathway in legume plants exposed to heavy metals. Part I. Effects of cadmium and lead on phenylalanine ammonia-lyase gene expression, enzyme activity and lignin content. Acta Biochim. Pol. 58 (2). https://doi.org/10.18388/abp.2011_2267.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E. 2011. Lead uptake, toxicity, and detoxification in plants. D.M. Whitacre (Ed.) Reviews of Environmental Contamination and Toxicology, 213, pp. 113−136. New York: Springer. https://doi.org/10.1007/978-1-4419-9860-6_4.

Qureshi, F.F., Ashraf, M.A., Rasheed, R., Hussain, I., Rizwan, M., Iqbal, M., Yong, J.W.H., 2024. Microbial-assisted alleviation of chromium toxicity in plants: a critical review. Plant Stress 11, 100394. https://doi.org/10.1016/j.stress.2024.100394.

Rafiq, M., Shahid, M., Shamshad, S., Khalid, S., Niazi, N.K., Abbas, G., Murtaza, B., 2018. A comparative study to evaluate efficiency of EDTA and calcium in alleviating arsenic toxicity to germinating and young *Vicia faba* L. seedlings. J. Soil Sed. 18 (6), 2271–2281. https://doi.org/10.1007/s11368-017-1693-5.

Rahman, M., Rahman, K., Sathi, K.S., Alam, M.M., Nahar, K., Fujita, M., Hasanuzzaman, M., 2021. Supplemental selenium and boron mitigate salt-induced oxidative damages in *Glycine* max L. Plants 10, 2224. https://doi.org/10.3390/plants10102224.

Redondo Gómez, S., Mateos Naranjo, E., Figueroa, M.E., Davy, A.J, 2010. Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*. Plant Biol. 12 (1), 79–87. https://doi.org/10.1111/j.1438-8677.2009.00207.x.

Rehman, M.Z., Rizwan, M., Ali, S., Ok, Y.S., Ishaque, W., Nawaz, M.F., Waqar, M., 2017. Remediation of heavy metal contaminated soils by using *Solanum nigrum*: a review. Ecotoxicol. Environ. Saf. 143, 236–248. https://doi.org/10.1016/j.ecoenv.2017.05.038.

Rehman, S., Abbas, G., Shahid, M., Saqib, M., Farooq, A.B.U., Hussain, M., Farooq, A., 2019. Effect of salinity on cadmium tolerance, ionic homeostasis and oxidative stress responses in *Conocarpus* exposed to cadmium stress: implications for phytoremediation. Ecotoxicol. Environ. Saf. 171, 146–153. https://doi.org/10.1016/j.ecoenv.2018.12.077.

Rahman, S.U., Han, J.C., Zhou, Y., Ahmad, M., Li, B., Wang, Y., Huang, Y., Yasin, G., Ansari, M.J., Saeed, M., Ahmad, I., 2024. Adaptation and remediation strategies of mangroves against heavy metal contamination in global coastal ecosystems: a review. J. Clean. Prod., 140868 p..

Rodríguez-Eugenio, N., McLaughlin, M., Pennock, D., 2018. Soil Pollution−A Hidden Reality. Food and Agriculture Organization of the United Nations, Rome.

Rosado, D., Usero, J., Morillo, J., 2016. Assessment of heavy metals bioavailability and toxicity toward *Vibrio fischeri* in sediment of the Huelva estuary. Chemosphere 153, 10–17. https://doi.org/10.1016/j.chemosphere.2016.03.040.

Rucińska-Sobkowiak, R., 2016. Water relations in plants subjected to heavy metal stresses. Acta Physiol. Plant. 38 (11), 1–13. https://doi.org/10.1007/s11738-016-2277-5.

Schück, M., Greger, M., 2023. Salinity and temperature influence removal levels of heavy metals and chloride from water by wetland plants. Environ. Sci. Pollut. Res. 30, 58030–58040. https://doi.org/10.1007/s11356-023-26490-8.

Shaghaleh, H., Azhar, M., Zia-ur-Rehman, M., Hamoud, Y.A., Hamad, A.A.A., Usman, M., Rizwan, M., Yong, J.W.H., Alharby, H.F., Al-Ghamdi, A.J., Alharbi, B.M., 2024. Effects of agro based organic amendments on growth and cadmium uptake in wheat and rice crops irrigated with raw city effluents: three years field study. Environ. Pollut. 344, 123365. https://doi.org/10.1016/j.envpol.2024.123365.

Shahid, M., Dumat, C., Khalid, S., Schreck, E., Xiong, T., Niazi, N.K., 2017. Foliar heavy metal uptake, toxicity and detoxification in plants: a comparison of foliar and root metal uptake. J. Hazard. Mater. 325, 36–58. https://doi.org/10.1016/j.jhazmat.2016.11.063.

Sharma, P., Dubey, R.S., 2005. Lead toxicity in plants. Braz. J. Plant Physiol. 17, 35–52. https://doi.org/10.1590/S1677-04202005000100004.

Shi, Q., Pang, J., Yong, J.W.H., Bai, C., Pereira, C.G., Song, Q., Wu, D., Dong, Q., Cheng, X., Wang, F., Zheng, J., Liu, Y., Lambers, H., 2020. Phosphorus-fertilisation has differential effects on leaf growth and photosynthetic capacity of *Arachis hypogaea* L. Plant Soil 447, 99–116. https://doi.org/10.1007/s11104-019-04041-w.

Shi, T., Ma, J., Wu, X., Ju, T., Lin, X., Zhang, Y., Li, X., Gong, Y., Hou, H., Zhao, L., Wu, F., 2018. Inventories of heavy metal inputs and outputs to and from agricultural soils: a review. Ecotoxicol. Environ. Saf. 164, 118–124. https://doi.org/10.1016/j.ecoenv.2018.08.016.

Shoukat, E., Aziz, I., Ahmed, M.Z., Abideen, Z., Khan, M.A., 2018. Growth patterns of *Phragmites karka* under saline conditions depend on the bulk elastic modulus. Crop Pasture Sci 69 (5), 535–545. https://doi.org/10.1071/CP17195.

Singh, V.K., Singh, R., Rajput, V.D., Singh, V.K., 2022. Halophytes for the sustainable remediation of heavy metal-contaminated sites: recent developments and future perspectives. Chemosphere 313, 137524. https://doi.org/10.1016/j. chemosphere.2022.137524.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., Savour, A., 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann. Bot. 115, 433–447. https://doi.org/10.1093/aob/mcu239.

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R., 2014. Abiotic and biotic stress combinations. New Phytol. 203, 32–43. https://doi.org/10.1111/nph.12797.

Swathika, M., Andal, N.M., Dharani, S., Singh, J., Pandey, S.S., Singh, K.R., Natarajan, A., 2024. Targeted elimination of heavy metals from industrial wastewater: synergistic effect of nano metal oxides. J. Taiwan Inst. Chem. Eng., 105485. https://doi.org/10.1016/j.jtice.2024.105485.

Taiz, L., Zeiger, E., 2015. Plant Physiology, 6th ed. Sinauer Associates, Inc, Sunderland, USA.

Tan, S.N., Yong, J.W.H., Ng, Y.F., 2010. Arsenic exposure from drinking water and mortality in Bangladesh. Lancet 376, 1641–1642. https://doi.org/10.1016/S0140-6736(10)62090-9.

Thakur, S., Sharma, S.S., 2016. Characterization of seed germination, seedling growth, & associated metabolic responses of *Brassica juncea L.* cultivars to elevated nickel concentrations. Protoplasma 253, 571–580. https://doi.org/10.1007/s00709-015-0835-0.

Tow, T.S.W., Eng, Z.X., Wong, S.P., Ge, L., Tan, S.N., Yong, J.W.H., 2018. *Axonopus compressus* (Sw.) Beauv.: a potential biomonitor for molybdenum in soil pollution. Int. J. Phytoremediat. 20, 1363–1368. https://doi.org/10.1080/15226514.2016.1207599.

Usman, K., Al-Ghouti, M.A., Abu-Dieyeh, M.H., 2019. The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant *Tetraena qatarensis*. Sci. Rep. 9 (1), 1–11. https://doi.org/10.1038/s41598-019-42029-9.

Van der Ent, A., Baker, A.J., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362 (1), 319–334. https://doi.org/10.1007/s11104-012-1287-3.

Van Oosten, M.J., Maggio, A., 2015. Functional biology of halophytes in the phytoremediation of heavy metal contaminated soils. Environ. Exp. Bot. 111, 135–146. https://doi.org/10.1016/j.envexpbot.2014.11.010.

Wang, Y., Qiao, M., Liu, Y., Zhu, Y., 2012. Health risk assessment of heavy metals in soils and vegetables from wastewater irrigated area, Beijing-Tianjin city cluster. China. J. Environ. Sci. 24 (4), 690–698. https://doi.org/10.1016/S1001-0742(11)60833-4.

Wiszniewska, A., Koźmińska, A., Hanus-Fajerska, E., Dziurka, M., Dziurka, K., 2019. Insight into mechanisms of multiple stresses tolerance in a halophyte *Aster tripolium* subjected to salinity and heavy metal stress. Ecotoxicol. Environ. Saf. 180, 12–22. https://doi.org/10.1016/j.ecoenv.2019.04.059.

Wu, D., Chen, C., Liu, Y., Yang, L., Yong, J.W.H., 2023. Iso-osmotic calcium nitrate and sodium chloride stresses have differential effects on growth and photosynthetic capacity in tomato. Sci. Hortic. 312, 111883. https://doi.org/10.1016/j.scienta.2023.111883.

Yildiztugay, E., Ozfidan-Konakci, C., Kucukoduk, M., 2014. The role of antioxidant responses on the tolerance range of extreme halophyte *Salsola crassa* grown under toxic salt concentrations. Ecotoxicol. Environ. Saf. 110, 21–30. https://doi.org/10.1016/j.ecoenv.2014.08.013.

Yong, J.W.H., Tan, S.N., Ng, Y.F., Low, K.K.K., Peh, S.F., Chua, J.C., Lim, A.A.B., 2010. Arsenic hyperaccumulation by *Pteris vittata* and *Pityrogramma calomelanos*: A comparative study of uptake efficiency in arsenic treated soils and waters. Water Sci. Technol. 61, 3041–3049. https://doi.org/10.2166/wst.2010.223.

Zhao, Q., Zhou, L., Liu, J., Cao, Z., Du, X., Huang, F., et al., 2018. Involvement of CAT in the detoxification of HT-induced ROS burst in rice anther and its relation to pollen fertility. Plant Cell Rep. 37, 741–757. https://doi.org/10.1007/s00299-018-2264-y.