

Tolerance to Zn toxicity in the halophyte *Lepidium latifolium* L. and the effect of salt on Zn tolerance and accumulation

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Tolerance to Zn toxicity in the halophyte *Lepidium latifolium* L. and the effect of salt on Zn tolerance and accumulation

Abstract: Halophytes exhibit a high cross-tolerance to multiple stresses that enable them to survive under harsh environmental conditions. We hypothesized that salt treatment in halophytes improves their tolerance against other stressors. To investigate the salt-mediated heavy metal tolerance in halophytes, *Lepidium latifolium* (Brassicaceae) was cultivated in the absence or presence of salt (100 mM NaCl) and excess Zn (200 μ M ZnSO₄), alone or in combination, for four weeks in the hydroponic medium. Salt treatment ameliorated the reduction of photosynthetic pigments in Zn-stressed plants and decreased Zn accumulation in the young leaves. The activity of peroxidase increased by both Zn toxicity and salt treatments; its maximum activity was achieved under the combination of both treatments associated with a significant reduction in malondialdehyde concentration. The activity of polyphenol oxidase increased by Zn stress alone or in combination with salt, accompanied by accumulation of free and cell wall-bound phenolics and enhanced lignin deposition in the leaves. Our results showed a mitigating effect of salt treatment in Zn-stressed plants through the activation of antioxidant defense and accumulation of phenolic compounds including flavonoids. Our results suggest *L. latifolium* as suitable species for revegetation and rehabilitation of saline soils contaminated with heavy metals.

Key words: halophytes; Zn toxicity; *Lepidium latifolium*; antioxidant defense; phenolics; lignin

Toleranca na strupenost Zn pri halofitu *Lepidium latifolium* L. in učinek soli na toleranco in kopičenje cinka

Izvleček: Halofiti imajo veliko navskrižno toleranco na multipli stres, kar jim omogoča preživetje v neugodnih okoljskih razmerah. Predpostavljamo, da obravnavanje s soljo pri halofitih izboljša njihovo toleranco na druge stresorje. Preučevali smo s soljo vzpodbujeno toleranco na težke kovine pri halofitu *Lepidium latifolium* (Brassicaceae), gojenem v prisotnosti ali odsotnosti soli (100 mM NaCl) in pribitku cinka (200 μ M ZnSO₄), posamično ali v kombinaciji, štiri tedne v hidroponskem gojišču. Obravnavanje s soljo je zmanjšalo upad vsebnosti fotosinteznih barvil v rastlinah v stresu zaradi cinka in zmanjšalo njegovo akumulacijo v mladih listih. Aktivnost peroksidaze se je povečala v obeh primerih, zaradi toksičnosti cinka in obravnavanja s soljo, in je dosegla največjo aktivnost v kombinaciji obeh obravnavanj, kar je bilo povezano z značilnim upadom koncentracije malondialdehida. Aktivnost polifenol oksidaze se je povečala v stresu zaradi cinka samega ali v kombinaciji z obravnavanjem s soljo, kar je bilo povezano z akumulacijo prostih ali na celično steno vezanih fenolov in pospešilo odlaganje lignina v listih. Ti rezultati so pokazali blažilni učinek obravnavanja s soljo v rastlinah v stresu zaradi cinka z aktiviranjem antioksidacijske obrambe in akumulacijo polifenolov. Rezultati tudi nakazujejo, da je halofit *L. latifolium* primerna vrsta za ozelenitev in izboljšanje slanosti tal onesnaženih s težkimi kovinami.

Ključne besede: halofiti; strupenost Zn; *Lepidium latifolium*; antioksidacijska obramba; fenoli; lignin

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1 INTRODUCTION

Environmental pollutants are rising progressively due to enormous economic development and the rapid growth of agriculture, urbanization, and industrial activities. Heavy metals are the most prevalent contaminants released from natural and anthropogenic sources into the environment and cause soil, air, and water pollution (Tchounwou et al., 2012). Heavy metals may accumulate in high concentrations in the edible part of crop plants, which are considered the primary cause of some diseases in humans and animals (Manara, 2012). Cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn) are the most common heavy metals that their accumulation in the environment causes an alarming situation of health problems (Tchounwou et al., 2012). DNA damage, inactivation of enzymes, and carcinogenic effects are predominant complications in humans after exposure to high concentrations of heavy metals (Manara, 2012).

Heavy metal toxicity in plants generally occurs through four principal mechanisms: 1) induction of oxidative stress through excess generation of reactive oxygen species (ROS) and changes in the permeability and integrity of the membranes, 2) changes in folding and activities of some proteins and enzymes due to the binding of heavy metals to their sulfhydryl groups, 3) competition with micronutrients to participate in cellular functions due to having similar physicochemical properties with them and 4) displacement of essential metal ion cofactors in the active sites of enzymes (Dal Corso, 2012).

Plants have some mechanisms for coping with heavy metal stress such as avoidance of metal uptake, prevention of their transport into the shoots, activation of defense mechanisms against ROS, and sequestration of heavy metals in the aerial parts through chelation by some organic compounds (Viehweger, 2014).

Zinc is an essential micronutrient for higher plants (Hafeez et al., 2013). However, similar to other heavy metals, it is toxic under excess concentrations (Küpper & Andresen, 2016). The toxic effects of Zn depend on its external bioavailable concentration, exposure time, and developmental stage of plants (Balaferj et al., 2020). Inhibition of shoot growth, reduction of root elongation, chlorosis of young leaves, and in some cases, cell death are the most obvious symptoms of Zn toxicity (Küpper & Andresen, 2016). Internal detoxification of Zn is achieved through its sequestration in the cytoplasmic compartments particularly in the vacuoles as chelated form with organic molecules, or as free ions (Balaferj et al., 2020).

Halophytes adapt to and grow under salinity conditions, consequently, they are interesting model species

for the study of adaptation and tolerance mechanisms in harsh environments. Numerous physiological and molecular adaptive mechanisms, e.g. the ability to limit the entry of ions into the transpiration stream, ion compartmentation, and synthesis of compatible osmolytes, have developed in the halophytes, that may confer also tolerance to toxic concentrations of heavy metals (Van Oosten & Maggio, 2014). Halophytes are considered potent candidates for removing heavy metals from soils due to their higher ability for accumulation and phytoremediation (Peng et al., 2022).

Lepidium L. is a genus belonging to Brassicaceae and encompasses over 175 species (Mummenhoff et al., 2009). *L. latifolium*, known as perennial pepperweed or tall white top, is a perennial facultative halophyte native to Asia and part of southeastern Europe (Spent, 2006). The plants spread through small and large numbers of seeds or vegetative reproduction and grow in a wide range of habitats. Because of its invasive and near-ubiquitous nature, this species is gaining more attention and is recognized as a global invader (Francis & Warwick, 2007). *L. latifolium* has also been widely used in traditional medicine as a diuretic and to reduce prostate hyperplasia, is a rich source of antioxidant compounds including phenolics, and is used as a whole herb for bacterial dysentery, enteritis, and other diseases (Kaur et al., 2013). Physiological studies on *L. latifolium* have mainly focused on drought resistance, salt tolerance, mineral elements, proteins, and amino acids (Hajiboland et al., 2020). There is no information on heavy metal accumulation or tolerance in this species.

Priming is a useful strategy for the improvement of the defense responses of plants against stressors. Various chemical compounds are used for priming; however, the effect of low concentrations of salt, as a priming agent has been relatively less investigated (Sako et al., 2020). In most studies, priming agents are applied at the seed germination stage, which may have a short-term effect on the tolerance of plants against stress. The impact of priming treatments in mature plants, however, has attracted much less attention from researchers.

Studies on the effect of low concentrations of salt in adult plants on their tolerance to heavy metal toxicity are still scarce. In addition, the effect of salt priming on heavy metal accumulation and tolerance in the halophytes has not been sufficiently addressed. A high cross-tolerance in the halophytes to multiple stresses that enables them to survive under extreme environmental conditions could be, at least partly, mediated by the salt-mediated induction of tolerance mechanisms. Our working hypothesis was that tolerance to toxic concentrations of Zn is enhanced through exposure to salt in the halophyte *L. latifolium*. For evaluation of the physiological response

of plants, the antioxidant defense system, activity of phenolics metabolizing enzymes, and lignin deposition were analyzed in addition to the elemental composition under Zn stress in the absence or presence of low salt concentration as priming treatment in this species.

2 MATERIALS AND METHODS

2.1 PLANTS CULTURE AND TREATMENTS

Seeds of *L. latifolium* were collected in 2016 from a wild-grown population in Meghan Playa in north central Iran. To obtain sufficient seeds with a high germination rate, the collected seeds were germinated and the young seedlings were cultivated in a private garden until flowering and seed set stage. The seeds from these plants were used for this work.

Seeds were surface-sterilized with 10 % sodium hypochlorite and sown in plastic containers filled with washed perlite and irrigated with distilled water. After three weeks, the young seedlings were transferred to the light and irrigated with 50 % Hoagland nutrient solution (pH 5.8). Four-week-old seedlings were cultivated in plastic containers filled with aerated 100 % Hoagland solution, and after one week, the seedlings were transplanted in 2-liter hydroponic pots for starting the treatments. Plants were grown in a growth chamber with 16/8 h of light/dark photoperiod at 25/17 °C, relative humidity of 50–60 %, and at a photon flux density of about 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by fluorescent lamps. The nutrient solutions were refreshed weekly.

To evaluate the tolerance level of plants to salt and Zn toxicity, a preliminary experiment was designed with 400 mM NaCl and 400 μM ZnSO₄ separately in the hydroponic medium. The concentration of salt and Zn in the culture media was gradually increased by adding 50 mM NaCl and 50 μM ZnSO₄ every day. One week after reaching the final concentration of salt and Zn, plants were harvested, and their biomass was determined.

In the main experiment, the five-week-old plants were pretreated with 100 mM salt (NaCl) for one week and then Zn (as ZnSO₄) treatment at 200 μM was used simultaneously with salt treatment. Both NaCl and Zn were applied increasingly, by 50 mM and 50 μM steps on daily basis, respectively. Plants were grown for four weeks under treatment conditions and then harvested. At harvest, plants were washed with distilled water, blotted dry on filter paper, and their fresh mass (FM) was determined.

2.2 BIOCHEMICAL AND ELEMENTAL ANALYSES

Shoot parts were separated into young (the second youngest leaf) and old leaves (the second oldest leaf), then were subjected to biochemical and elemental analyses. The activity of enzymes was determined in fresh samples immediately after harvest. Other biochemical analyses were carried out after storage at –20 °C for a maximum of six days. Oven-dried samples were weighed and then used for elemental analyses.

2.2.1 Pigments concentration

Photosynthetic pigments including chlorophylls (Chl) and carotenoids were extracted from the leaves in ice-cold 80 % acetone in the dark at 4 °C. The absorbance of extracts was determined at 470, 645, and 662 nm, and the concentration of pigments was calculated and expressed based on leaf fresh weight (Lichtenthaler & Welburn, 1983). The flavonoids content was determined in the leaf homogenate prepared in an extracting solution containing 2 % AlCl₃ in methanol. After centrifugation, the absorbance of supernatants was measured at 415 nm. The total flavonoids concentration was expressed as μg quercetin equivalent per g of FW by drawing a calibration curve with 0–16 mg l⁻¹ concentration of authentic quercetin (Arvouet-Grand et al., 1994).

2.2.2 Antioxidant enzymes assay

The total activity of superoxide dismutase (SOD, EC 1.15.1.1) in the plant samples was assayed using the mono-formazan formation test. One unit of SOD activity was defined as the amount of enzyme required for a 50 % inhibition in NBT (ρ -nitro blue tetrazolium chloride) reduction through the monitoring of the changes in the absorbance at 560 nm, compared to the control samples without the enzyme aliquot (Giannopolitis & Ries, 1977). Ascorbate peroxidase (APX, EC 1.11.1.11) activity in the extracts was calculated by determining the decrease in the absorbance at 290 nm for 2 min due to the oxidation of ascorbic acid using an extinction coefficient of 2.8 mM⁻¹ cm⁻¹ (Boominathan & Doran, 2002). The activity of CAT in the extracts was estimated by monitoring the decreases in the absorbance of H₂O₂ at 240 nm for 2 min. One unit of activity was defined as the quantity of enzyme needed to decompose 1 μmol H₂O₂ per min, us-

ing the extinction coefficient of $0.28 \text{ mM}^{-1} \text{ cm}^{-1}$ (Chance & Maehly, 1954). The activity of peroxidase (POD, EC 1.11.1.7) was assessed in a reaction mixture using guaiacol as substrate, and the enzyme activity was measured at 470 nm using extinction coefficient ($26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) of tetraguaiacol (Ranieri et al., 2001).

The concentration of total soluble proteins was determined using the Bradford assay (Bradford, 1976) and bovine serum albumin (BSA, Merck) as the standard.

2.2.3 H_2O_2 , malondialdehyde (MDA), and proline concentrations

The concentration of H_2O_2 in the extracts was determined by recording the absorbance at 390 nm and using the plotted calibration curve in the range of 0–120 μM H_2O_2 (Harinasut et al., 2003). The concentration of malondialdehyde (MDA) as a marker of lipid peroxidation in the leaf extracts was determined by the absorbance at 532 nm due to its reaction with thiobarbituric acid and using a plotted standard curve with 1,1,3,3-tetraethoxypropane (Hodges et al., 1999). For the estimation of proline, leaf samples were extracted in sulfosalicylic acid, and the supernatants were used for the determination of proline according to the method of Bates et al. (1973) and with ninhydrin as a reagent.

2.2.4 Total phenolics concentration and lignin quantification

Phenolic compounds were extracted in 70 % aqueous methanol three times, and after centrifugation, the supernatant was used for the determination of soluble phenolics, while cell wall-bound phenolics and lignin were quantified in the pellet. For the release of the cell wall (CW)-bound phenolics, the pellet was washed consecutively with water and Triton X-100, then after incubation with 20 mM NH_4 -oxalate (70 °C) followed by 100 mM NaOH for 24 h, the phenolics were released from the CW. The concentrations of soluble and CW-bound phenolics were determined using Folin–Ciocalteu reagent at 765 nm and gallic acid as standard (Swain & Hillis, 1959). After air-drying, the residual CW fraction was used for lignin extraction and determination using the acetyl bromide method by recording the absorbance at 280 nm and a specific absorption coefficient value of $8.4 \text{ l g}^{-1} \text{ cm}^{-1}$ (Morrison, 1972).

2.2.5 Phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) activity

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity in the leaf and root samples were evaluated as the amount of the formed trans-cinnamic acid at 290 nm and was calculated using its extinction coefficient of $9630 \text{ mM}^{-1} \text{ cm}^{-1}$ (Dickerson et al., 1984). Polyphenol oxidase (PPO, EC 1.14.18.1) activity was determined according to the method described by Casado-Vela et al. (2005). The changes in the absorbance at 334 nm due to the oxidation of pyrogallol were used for the calculation of the PPO activity in the extracts.

2.2.6 Mineral analyses

The oven-dried leaf and root samples were used for mineral analyses. The samples were ashed in a muffle furnace at 550 °C for 8 h, and after dissolving in 10 % HCl and filtration, were made to volume with distilled water. The concentrations of Na, K, and Ca were determined by flame photometry (PFP7, Jenway, UK), and the standard solutions of the examined elements were used for the construction of the calibration curves. The concentration of Zn in the samples was analyzed by atomic absorption spectrophotometry (AA-6300, Shimadzu, Japan).

2.2.7 Experimental design and statistical analyses

This experiment was performed using a complete randomized block design with four independent replicates per treatment. Data were presented as mean \pm standard deviation (SD). The comparison of means was carried out using SigmaStat 3.5 (Systat Software Inc., USA) with Tukey's test at $p < 0.05$.

3 RESULTS

According to the results of the preliminary experiment, the growth of *L. latifolium* plants was decreased under both salinity and Zn toxicity treatments. Reduction of growth upon exposure to 400 mM salt was 49 % and 52 % for the fresh mass (FM) and dry mass (DM) of the shoots, respectively. The reduction of FM and DM of aerial parts under the effect of 400 μM Zn was 50 % and 43 %, respectively (Table 1).

Table 1: Decline in biomass production (% over control) in *L. latifolium* grown with salt (400 mM NaCl) or toxic Zn concentration (400 μ M ZnSO₄) for three weeks in a hydroponic medium

	Reduction of FM	Reduction of DM
Salinity	49 \pm 6	52 \pm 8
Zn toxicity	50 \pm 13	43 \pm 18

The main experiment was conducted to study salt, Zn, and their interaction effects on the growth, biochemical and mineral attributes of the plants as described below.

3.1 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON PLANTS BIOMASS AND CONCENTRATION OF PIGMENTS

Due to a strong reduction of growth in the preliminary experiment, lower concentrations of salt and Zn were applied in the main experiment. According to the obtained results, the shoot DM was decreased by salt, Zn toxicity, and especially under a combination of both treatments (Figure 1A). Root DM decreased significantly under Zn toxicity alone or in combination with salt, while it was not significantly affected by salinity as a single treatment (Figure 1B).

The leaf concentration of Chl was decreased by all applied treatments. The highest reduction (90 %) was observed under Zn toxicity without salt and the lowest decline (17 %) was found under salt as a single treatment (Table 2). Unlike Chl, the leaf concentration of carotenoids increased due to salinity treatment but significantly decreased by Zn toxicity alone or in combination with salt. The leaf concentration of flavonoids decreased by both salt and Zn toxicity as single stresses, while it was significantly higher than the control plant in the combinative treatment (Table 2).

3.2 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON THE ACTIVITY OF ANTIOXIDANT ENZYMES AND CONCENTRATION OF RELATED METABOLITES

The activity of SOD decreased by salt treatment alone or in combination with Zn toxicity while remaining unaffected under Zn toxicity as a single treatment (Figure 2A). The activity of CAT showed a significant reduction under the combined treatment but remained unchanged under the individual treatments of either salt or Zn toxicity (Figure 2B). The activity of APX, in con-

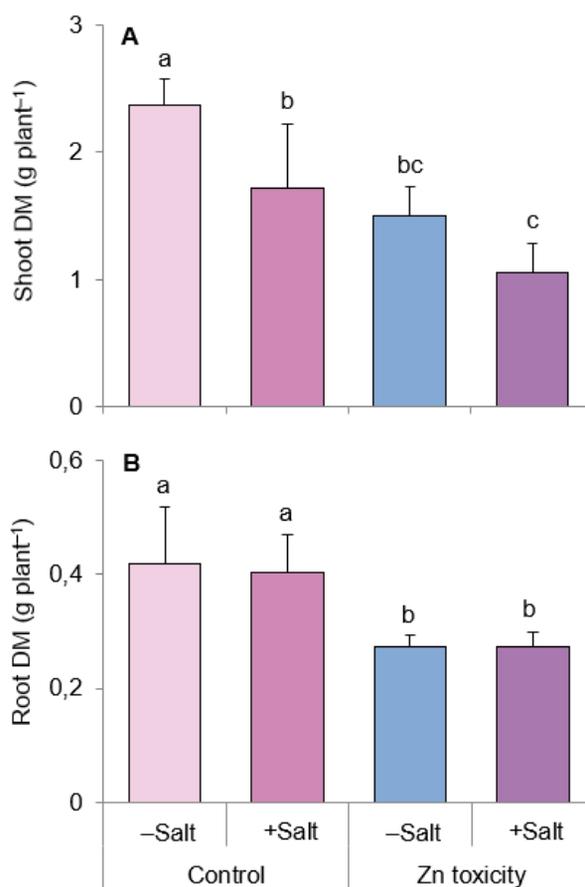


Figure 1: The biomass of shoots (A) and roots (B) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 μ M as ZnSO₄). Bars indicated by different letters are significantly different ($p < 0.05$)

trast, increased by salt treatment alone or in combination with Zn toxicity. The effect of the latter treatment as single stress on the APX activity was not significant (Figure 2C). The activity of POD was significantly increased by salinity and Zn toxicity; the highest enzyme activity was observed under a combination of these treatments (Figure 2D).

The concentration of H₂O₂ was increased by all applied treatments. The effect of salinity alone or in combination with Zn toxicity was significantly higher than that of Zn toxicity as single stress (Figure 3A). The concentration of MDA increased under salinity and Zn toxicity as single stress. Under the combination of both treatments, however, this parameter did not differ from that in the control plants (Figure 3B). The concentration of proline showed a significant increase under the influence of all applied treatments. The effect of Zn toxicity as a single treatment, however, was higher than that of its combination with salinity; the lowest effect was observed upon salt as single stress (Figure 3C).

Table 2: The leaf concentrations of chlorophylls (Chl a+b), carotenoids (mg g⁻¹ FM), and flavonoids (µg g⁻¹ FM) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as ZnSO₄). Data of each column indicated by the different letters are significantly different ($p < 0.05$)

Treatment		Chl (a+b)	Carotenoids	Flavonoids
Control	-Salt	2.06 ± 0.10 ^a	0.16 ± 0.01 ^b	88 ± 5.8 ^b
	+Salt	1.70 ± 0.19 ^b	0.32 ± 0.01 ^a	66 ± 2.6 ^c
Zn toxicity	-Salt	0.19 ± 0.06 ^d	0.08 ± 0.02 ^d	78 ± 5.7 ^{bc}
	+Salt	1.32 ± 0.02 ^c	0.12 ± 0.01 ^c	147 ± 14.0 ^a

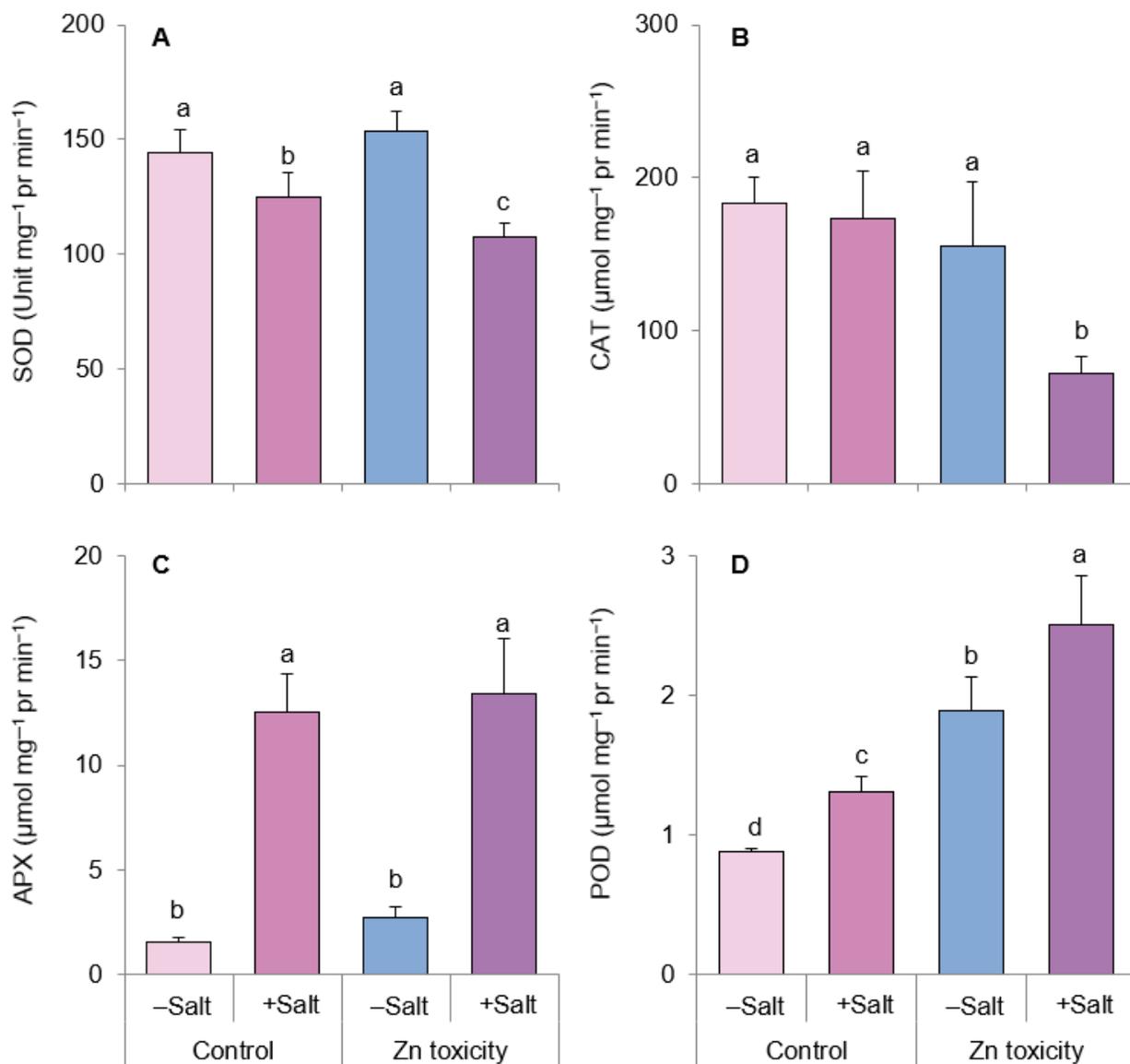


Figure 2: The leaf activity of superoxide dismutase (SOD) (A), catalase (CAT) (B), ascorbate peroxidase (APX) (C), and peroxidase (POD) (D) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as ZnSO₄). Bars indicated by different letters are significantly different ($p < 0.05$)

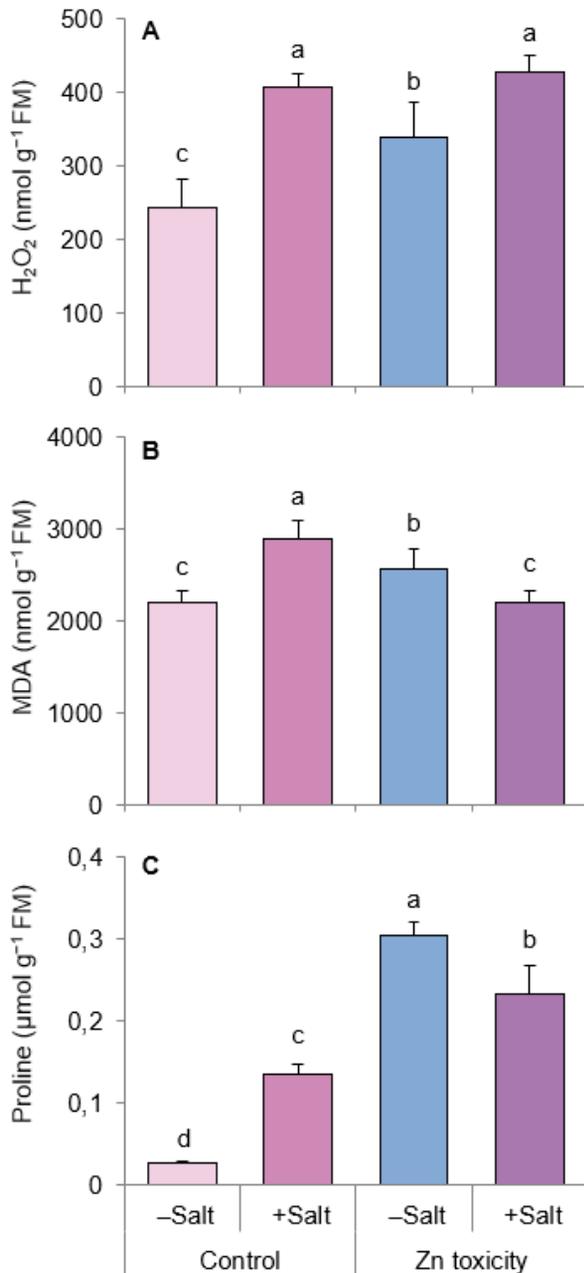


Figure 3: The leaf concentration of hydrogen peroxide (H_2O_2) (A), malondialdehyde (MDA) (B), and proline (C) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 μ M as $ZnSO_4$). Bars indicated by different letters are significantly different ($p < 0.05$)

3.3 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON THE ACTIVITY OF PAL, PPO, AND THE CONCENTRATIONS OF PHENOLICS AND LIGNIN

The leaf activity of PAL was decreased by all ap-

plied treatments without difference among the three treatments. The root activity of this enzyme, in contrast, remained unchanged or rather increased. The latter effect was observed under Zn toxicity as a single treatment (Figures 4A and 4B). The leaf activity of PPO increased under Zn toxicity alone or in combination with salinity, while salt treatment as a single stress did not affect this parameter (Figure 4C). In the roots, however, all applied treatments increased the PPO activity; the effect of Zn toxicity alone was significantly higher than that of the combinative treatment (Figure 4D).

The concentration of free phenolics decreased under salt stress, while increased by Zn toxicity treatment, and remained unaffected under the combination of both treatments (Figure 5A). The concentration of CW-bound phenolics, in contrast, increased by salt stress either alone or in combination with high Zn concentration while was not affected by Zn toxicity as a single treatment (Figure 5B). The lignin concentration increased by salt, Zn toxicity, and their combination without difference among the three treatments (Figure 5C).

3.4 THE EFFECT OF SALT, ZN, AND THEIR COMBINATION ON THE CONCENTRATION OF ELEMENTS IN THE LEAVES AND ROOTS

As expected, Zn was accumulated in the leaves and roots of plants treated with a high Zn concentration. In the young leaves, the concentration of Zn was significantly lower under the combinative treatment than that under Zn as a single treatment. The effect of salt on the reduction of Zn concentration, however, was not observed in the old leaves and roots (Figure 6).

Also, as anticipated, Na was accumulated in the young and old leaves and roots upon exposure to salt in the medium. Leaf accumulation of Na in plants treated with a combination of salt and Zn toxicity was significantly higher than that in plants grown with salt as a single treatment. The opposite was observed in the roots; Na concentration in this organ was lower under combinative treatment compared to that under single salt treatment (Table 3).

The concentration of K decreased significantly by salt treatment in the old and young leaves and in the roots. Treatment of plants with toxic Zn concentration caused also a decrease in the K concentration of old leaves and roots but did not affect this parameter in the young leaves. Reduction of K concentration was also observed under the combination of Zn stress and salt treatments. However, the effect of combinative treatment on

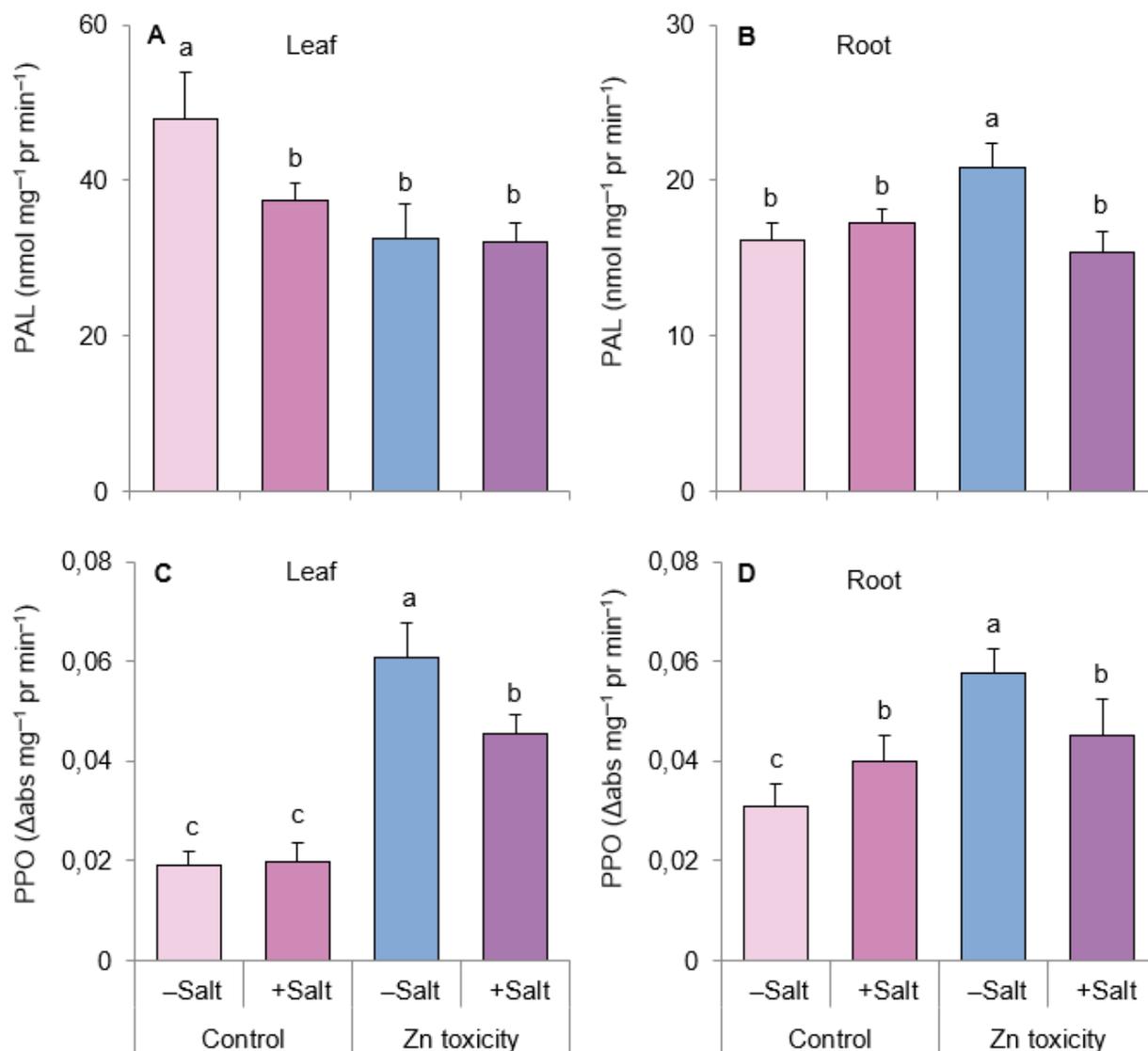


Figure 4: The activity of phenylalanine ammonia-lyase (PAL) (A and B) and polyphenol oxidase (PPO) (C and D) in the leaves and roots of *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 μM as ZnSO₄). Bars indicated by different letters are significantly different ($p < 0.05$)

the leaf K concentration was less than the effect of single salt stress (Table 3).

The effect of salt and Zn toxicity treatments on the Ca concentration was dependent on the plant organ. The presence of salt in the medium increased Ca concentration in the roots and did not influence it in the young leaves while decreasing it in the old leaves. Treatment with toxic Zn concentration led to an increase in the Ca concentration of the young leaves but resulted in its reduction in the old leaves and roots. The effect of combinative treatment in the reduction of Ca concentration was observed only in the old leaves and roots (Table 3).

4 DISCUSSION

Studies on the stress tolerance in Brassicaceae species, particularly its halophyte members, attract the attention of plant scientists because of the possibility for comparison of these species with the model plant, *Arabidopsis thaliana* (L.) Heynh.. Based on these studies, *Schrenkiella parvula* (Schrenk) D.A.German & Al-Shehbaz (Hajiboland et al., 2018), *Cakile maritima* Scop. (Debez et al., 2004), and *Thellungiella salsuginea* (Pall.) O.E.Schulz (syn. *Eutrema salsugineum* (Pall.) Al-Shehbaz & Warwick) (Gao et al., 2008) are the most salt-tolerant Bras-

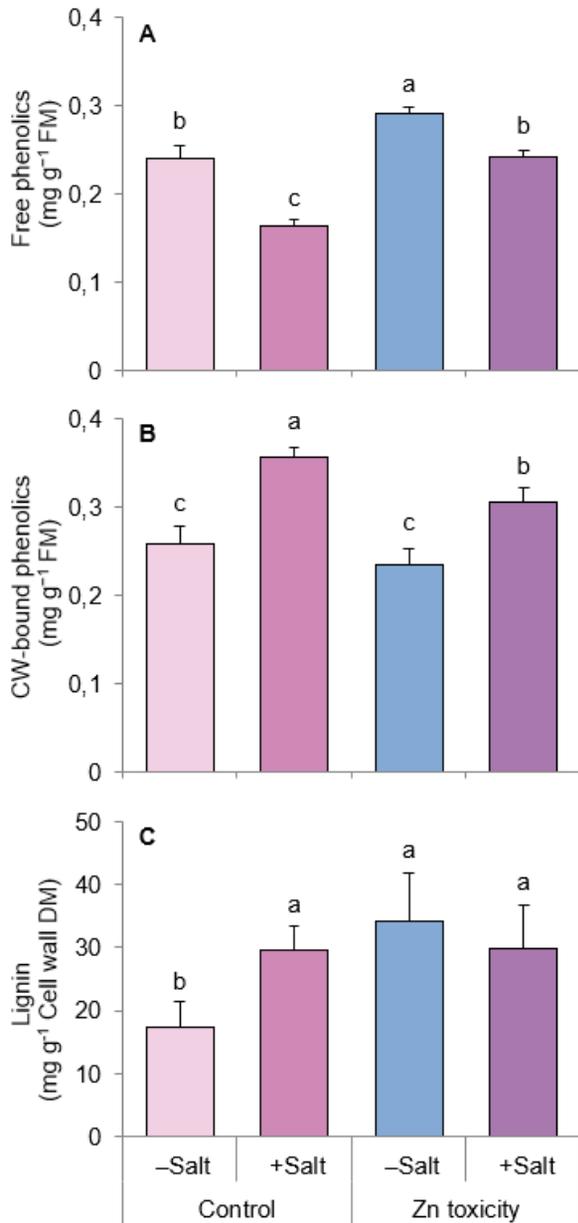


Figure 5: The leaf concentration of free phenolics (A), cell wall (CW)-bound phenolics (B), and lignin (C) in *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 μ M as ZnSO₄). Bars indicated by different letters are significantly different ($p < 0.05$)

sicaceae species, which are able to grow even under salt concentrations of about 400 mM. The tolerance mechanisms in halophytes of this family include avoidance of excessive uptake of Na and maintenance of a proper ratio of K/Na in the cytosol, accumulation of organic osmolytes (including proline), activation of defense against ROS as well as changes in the levels of plant hormones (Van Zelm et al., 2020). To expand our knowledge of the

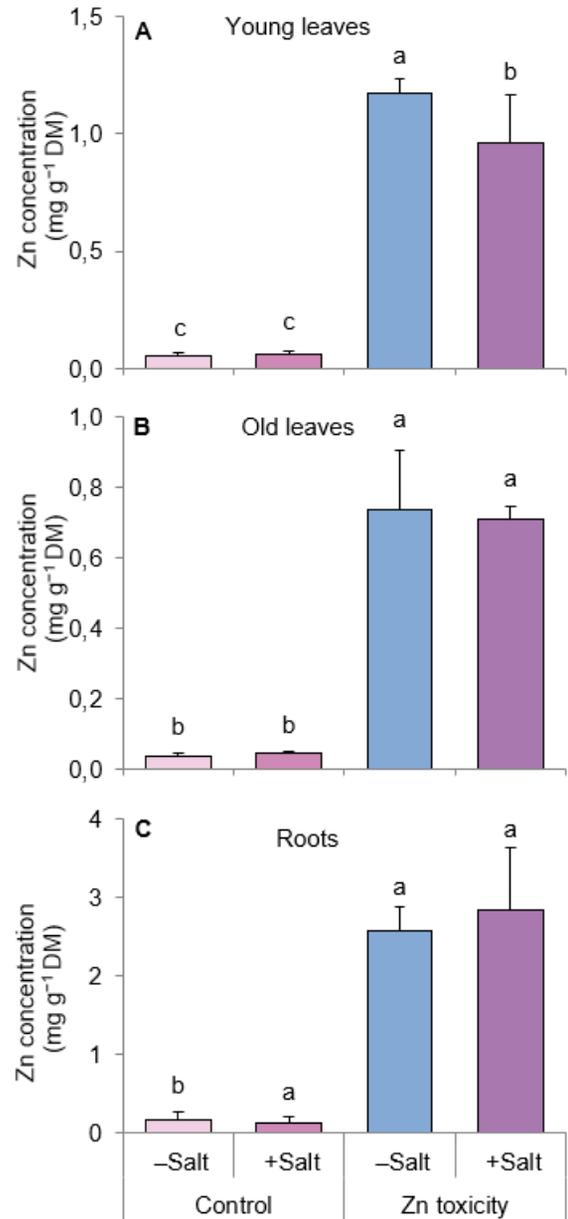


Figure 6: Zn concentration in the young leaves (A), old leaves (B), and roots (C) of *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 μ M as ZnSO₄). Bars indicated by different letters are significantly different ($p < 0.05$)

mechanisms of salt tolerance and to find other model plants within Brassicaceae, i.e. *Arabidopsis*-related model species (ARMS, Arbelet-Bonnin et al., 2019), more investigations are necessary particularly on the halophytes of this family. In this study, *L. latifolium*, a facultative halophyte species was investigated for salt tolerance and its interaction with plant response to Zn toxicity.

Table 3: Concentrations (mg g⁻¹ DM) of Na, K, and Ca in the young leaves, old leaves, and roots of *L. latifolium* grown for three weeks in the absence or presence of salt (100 mM NaCl) and Zn (200 µM as ZnSO₄). Data of each column within each organ indicated by different letters are significantly different (*p* < 0.05)

Treatment		Na	K	Ca
		Young leaf		
Control	-Salt	0.6 ± 0.2 ^c	43 ± 0.5 ^a	35 ± 3 ^b
	+Salt	6.5 ± 1.6 ^b	32 ± 2.4 ^c	38 ± 7 ^b
Zn toxicity	-Salt	1.5 ± 0.7 ^c	41 ± 2.8 ^a	49 ± 2 ^a
	+Salt	15 ± 2.8 ^a	35 ± 1.6 ^b	37 ± 9 ^b
Old leaf				
Control	-Salt	2.3 ± 1.0 ^c	68 ± 2.0 ^a	66 ± 7 ^a
	+Salt	18 ± 3.0 ^b	34 ± 1.0 ^d	44 ± 9 ^c
Zn toxicity	-Salt	2.4 ± 0.6 ^c	53 ± 5.0 ^b	58 ± 6 ^b
	+Salt	42 ± 6.0 ^a	41 ± 3.6 ^c	45 ± 3 ^c
Root				
Control	-Salt	1.1 ± 0.3 ^c	91 ± 8.7 ^a	62 ± 11 ^b
	+Salt	14 ± 2.0 ^a	44 ± 7.9 ^c	154 ± 13 ^a
Zn toxicity	-Salt	1.2 ± 0.1 ^c	78 ± 2.6 ^b	44 ± 9 ^c
	+Salt	6.5 ± 1.2 ^b	38 ± 1.7 ^c	23 ± 5 ^d

4.1 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON PLANT BIOMASS

In comparison to glycophytes, halophytes have generally higher resistance against not only salt but also other soil-derived abiotic stressors, such as drought and heavy metal toxicity (Lokhande & Suprasanna, 2012). Mechanisms such as heavy metal exclusion, reduction of their mobility in soil (MacFarlane & Burchett, 2002), restriction of shoot-root translocation (Mejías et al., 2013), internal detoxification and sequestration, activation of the antioxidant system (Sharma et al., 2016), and even excretion of heavy metals through salt glands and trichomes (Lokhande & Suprasanna, 2012) all contribute to a higher heavy metal tolerance in halophytes. In addition, salt treatment mitigates heavy metal toxicity effects in some halophytes. The biomass of *Sesuvium portulacastrum* (L.) L. under Cd toxicity (Ghnaya et al., 2007) and growth of *Mesembryanthemum crystallinum* L. (Kholodova et al., 2005) and *Spartina densiflora* Brongn. (Mahon & Carman, 2008) under Zn toxicity were higher under simultaneous application of salt and heavy metal toxicity compared to single heavy metal stress. In our study, however, the effect of Zn toxicity was not mitigat-

ed by simultaneous treatment with salt suggesting that the ameliorative effect of salt on heavy metal toxicity in the halophytes is not common and is likely dependent on the species and the heavy metal. To the best of our knowledge, the effect of salt on heavy metal toxicity has not been investigated in the Brassicaceae halophytes, but in the glycophytes of this family including *Brassica napus* L. and *Brassica juncea* (L.) Czern. the combined treatment of salt and Cd had a higher inhibitory effect on the growth and photosynthesis of these species compared to single treatments (Shah et al., 2011).

4.2 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENT OF SALT AND ZN TOXICITY ON THE CONCENTRATIONS OF LEAF PIGMENTS

An antagonistic effect of toxic heavy metal concentrations on the uptake and utilization of Fe, the main element for Chl biosynthesis, has been well documented (Leiková et al., 2017). In addition, impairment of biochemical reactions under heavy metal toxicity leads to the formation of excess excitation energy and generation of ROS that in turn cause instability of the thylakoid membranes and destruction of Chl (Riyazuddin et al., 2021). In *L. latifolium*, the leaf concentration of Chl was decreased upon exposure to excess Zn by up to 90%. This was probably the consequence of both reductions in biosynthesis and the rise of its destruction.

Carotenoids play important roles in the stability of chloroplast membranes and protect photosynthetic apparatus against damages caused by excess excitation energy (Uarrota et al., 2018). A significant increase in the leaf carotenoids observed in salt-treated *L. latifolium* indicates that some protection mechanisms are triggered under these conditions leading to a reduction of injury to the chloroplasts similar to that reported for other halophytes, *Arthrocnemum macrostachyum* (Moric.) Pirainen & G.Kadereit, *Sarcocornia fruticosa* (L.) A.J.Scott (Ghanem et al., 2021), and *Nitraria retusa* (Forssk.) Asch. (Boughalleb & Denden, 2011). However, a salt-mediated increase in the leaf carotenoids has not been observed in all halophytes, as in *Salicornia europaea* L. salt treatment reduced this parameter (Aghaleh et al., 2009). Contrary to salt treatment, leaf carotenoids significantly decreased under Zn toxicity in our study, which may increase the vulnerability of photosynthetic apparatus to ROS-induced damages under these conditions. In *Halimione portulacoides* (L.) Aellen the amounts of carotenoids increased under Zn toxicity (400 mM) (Cambrollé et al., 2012) while in another halophyte, *Avicennia marina*

(Forssk.) Vierh. the amount of this pigment decreased under excess Cu and Zn, but remained unchanged under the Pb toxicity (MacFarlan & Burchett, 2002).

The leaf concentrations of Chl, carotenoids, and flavonoids were significantly higher under the combination of salt with Zn toxicity compared to the single Zn treatment in *L. latifolium*, likely as the consequence of the effect of salt treatment on the stimulation of protection mechanisms in the chloroplasts. Application of low concentrations of salt protects the structure and function of chloroplasts; ascorbate plays a central role in this priming effect because *Arabidopsis* mutants lacking ascorbate show a considerable disruption of photosynthesis under salt stress (Acosta-Motos et al., 2017). Similarly, salt priming decreased high temperature-induced damage to the photosystem II in *Atriplex centralasiatica* Iljin (Qiu & Lu, 2003). In *Suaeda salsa* (L.) Pall. salt priming increased the quantum yield of photosystem II and increased the amount of unsaturated fatty acids (Cheng et al., 2014). Flavonoids are low-molecular-weight polyphenolic metabolites not only involved in ROS scavenging, but also as chelating molecules, bind to heavy metals and thus, play a role in internal detoxification (Keilig & Ludwig-Müller, 2009; Samanta et al., 2011). Under the combination of salt and Zn treatments, the leaf concentration of flavonoids was two-fold higher than that found under control and single-stress conditions in *L. latifolium*. Enhanced flavonoids level under combinative treatment was associated with significantly lower MDA concentration suggesting their contribution to the prevention of membrane damage under these conditions.

4.3 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON THE FUNCTION OF THE ANTIOXIDANT DEFENSE SYSTEM

Higher constitutive or stress-induced antioxidant defense is one of the most important mechanisms in halophytes to cope with various environmental stresses (Sruthi et al., 2017). SOD is involved in scavenging superoxide radicals as one of the most damaging ROS in cells (Sruthi et al., 2017). In this study, however, the activity of SOD decreased under both single and combinative salt and Zn stresses. Reduction in the activity of SOD under salinity has also been reported in other halophytes such as *Gypsophila oblancoolata* Barkoudah (Sekmen et al., 2012) and *Salvadora persica* L. (Rangani et al., 2016).

Unlike SOD, the activity of APX was significantly increased by salt treatment alone or in combination with Zn toxicity, which is in agreement with many reports on the effect of salinity on the APX activity in the glycophytes,

e.g. *Arabidopsis thaliana* and halophytes, e.g. *Cakile maritima* (Ellouzi et al., 2011) and *Sesuvium portulacastrum* (Ben Amor et al., 2020). The activity of CAT was not affected by the single salt and Zn toxicity stress and significantly decreased under combinative treatments. The lack of any response to low concentrations of salt (100 mM) was similar to another report in the euhalophyte, *Salvadora persica*, where the activity of CAT did not change under 250 mM salt, but increased under 500 mM salt treatment (Rangani et al., 2016). Unlike CAT and APX, the activity of POD increased by all three applied treatments and could probably be considered as an indicator of the effect of treatments. Peroxidases are a large group of enzymes oxidizing a wide array of substrates using H_2O_2 (Veitch, 2004). Several reports showed a consistent increase in the activity of POD by salt stress both in the glycophytes and in halophytes (Yang et al., 2010; Ellouzi et al., 2011). The highest activity of POD was observed under combinative treatment associated with a reduction of MDA to the levels observed in control plants. Overall, our results demonstrated that antioxidant enzymes respond differently to the applied treatments depending on enzyme and stress factors, and suggest the different contributions of each enzyme in the defense of plants against salt, heavy metals, and their combinations.

H_2O_2 plays a dual role in plant stress response: it acts as a signaling molecule at the nanomolar or low micromolar range of concentration (per g FM) while at the millimolar level damages the molecular structure of proteins, lipids, and nucleic acids (Černý et al., 2018). The range of cytosolic H_2O_2 concentration in our plants (200-400 nmol g⁻¹ FM, Figure 3A) was highly relevant to its signaling function. Furthermore, the ability of salt treatment in the induction of H_2O_2 signal was higher than that of Zn toxicity, leading in turn to a higher H_2O_2 level in the combinative treatment (Figure 3A). This may be one of the mechanisms for the ameliorative effect of salt on Zn stress in this work, as was also reflected in the levels of biochemical stress markers under combinative treatment. The effect of salt on the induction of H_2O_2 signaling and stress tolerance has been observed in other halophytes (Ellouzi et al., 2011). Interestingly, H_2O_2 signaling in a halophyte (*L. latifolium*) is characteristically different from its glycophyte relative (*Lepidium sativum* L.) in the timing and magnitude of induction (Hajiboland et al., 2020).

Accumulation of MDA, as the final product of peroxidation of poly-unsaturated fatty acids in the membranes, is one of the most common effects of heavy metal toxicity in plants (Manara, 2012). An increase in the MDA content has been reported in *Atriplex rosea* L. and *Arabidopsis hortensis* L. grown in soils contaminated with Ni and Zn (Kumari et al., 2019) or in *Acanthus ilici-*

folius L. under Cd stress (Shakira & Puter, 2019). In our work, the MDA concentration increased by Zn toxicity as a single treatment, while was reduced to the level of that in control plants under the combination of Zn with salt treatment. This may indicate higher protection of cell and plastid membranes in the combinative treatment, as was also reflected in the higher concentration of leaf pigments under these conditions compared with Zn toxicity as a single treatment (Table 2). The protective role of salt under heavy metal stress has also been observed in the halophyte *Atriplex halimus* L. against Cd and Cu toxicities as a reduction of MDA accumulation in the roots under the combination of heavy metals with salt treatments (Bankaji et al., 2016).

Accumulation of proline driven by different environmental stresses is a well-documented response in plants (Hayat et al., 2012). The major function of proline under salt stress is osmotic regulation, which along with other compatible organic osmolytes, e.g. polyols and glycine betaine, confronts the osmotic component of salt stress (Siddique et al., 2018). The function of proline, however, is not limited to an osmotic role but it contributes to a wide range of protective functions, including stimulation of antioxidant defense enzymes, role in the stability of protein structures, and redox homeostasis (Hayat et al., 2012). Under heavy metal stress, proline protects cells against toxicity damage (Siddique et al., 2018). In the halophyte *Acanthus ilicifolius*, proline concentration increased in response to Cd toxicity (Shakira & Puthur, 2019) and in *Mesembryanthemum crystallinum* under excess Zn concentration (Kholodova et al., 2005). The expression of the proline biosynthetic gene (P5CS) was increased under Cr toxicity and its combination with salt in *Chenopodium quinoa* Willd. that was associated with proline accumulation in this species (Guarino et al., 2020). In our work, the leaf concentration of proline increased under salt stress, particularly by Zn toxicity treatment. Although the proline level was lower under combinative treatment compared to single Zn stress, it remained still higher than that found under salt stress and was about 9-fold higher than the proline concentration of control plants. As an indicator of stress (Ashraf & Harris, 2004), lower proline concentration in the combinative treatment could be likely the result of mitigation of the Zn toxicity stress, as was also reflected in the lower MDA content and higher amounts of leaf pigments. In agreement with our findings, the proline concentration in the halophyte *Kosteletzkya pentacarpos* (L.) Ledeb. increased under Cd toxicity, while decreased under the combination of salt and Cd stress (Zhou et al., 2019).

4.4 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON THE CONCENTRATIONS OF PHENOLICS, LIGNIN, AND THE ACTIVITY OF RELATED ENZYMES

Phenolic compounds possessing an aromatic ring with one or more hydroxyl substituents are contributed to ROS scavenging and stabilization of membranes and other cell structures (Moura et al., 2010). An enhanced synthesis and accumulation of phenolic compounds under the toxicity of heavy metals has been extensively reported (Ghori et al., 2019). In *Kandelia obovata* Sheue, Liu & Yong, Cd toxicity caused phenolics accumulation accompanied by a significant increase in PPO activity (Chen et al., 2019). Similarly, in *Matricaria chamomilla* L., the concentration of phenolic compounds increased under Ni toxicity associated with an increase in the activity of PAL but a decrease in the activity of PPO (Kováčik et al., 2009). In our work here, the concentration of free phenolics increased under Zn toxicity treatment (Figure 5A), indicating a probable role for phenolics in the increasing plants' resistance against Zn toxicity. In addition to their antioxidant and protective function, a metal chelating capacity of phenolics as a mechanism for the internal detoxification of heavy metals has been documented (Michalak, 2006). The capacity of particular phenolics such as cinnamic acid, ferulic acid, gallic acid, and naringenin for Zn chelation has been demonstrated both *in vivo* and *in vitro* (Fedenko et al., 2022).

In addition to free phenolics, low molecular weight phenolic acids that are bound to various CW components have several important roles including responses to stresses (Wallace & Fry, 1994). The concentration of CW-bound phenolics was higher in the salt-treated plants either as single stress or in combination with Zn toxicity (Figure 5B). The carboxylic groups of CW-bound phenolics have a high affinity for heavy metals and the formed complexes show high stability constants (McDonald et al., 1996). This mechanism may contribute to Zn detoxification in our work, particularly in the combinative treatment with an enhanced concentration of CW-bound phenolics.

The composition of plant CWs is modified under biotic and abiotic stresses (Gall et al., 2015). The biosynthesis of lignin, as one of the major components of the CW, increases under various stresses, including salinity and heavy metal toxicity (Moura et al., 2010). ROS produced under stress in the apoplasts participate in lignin synthesis via various enzymes including POD and PPO (Ali

et al., 2006). In *Arabidopsis thaliana*, salt treatment up-regulates laccase- and POD-encoding genes leading to lignification in salt-stressed plants (Chun et al., 2019). In *Tamarix hispida* Willd., the expression of genes involved in the lignin biosynthesis, i.e. S-adenosyl methionine synthase (SAM synthase) and catechol-O-methyltransferase (COMT) were increased under salt stress (Han et al., 2022). Interestingly, there is a relationship between the extent of lignification and salt tolerance, so that leaf lignification under salt stress was higher in the halophyte *L. latifolium* than that was found in its glycophyte relative, *L. sativum* (Hajiboland et al., 2020). In this work, the lignin concentration was increased by all applied treatments, especially by Zn toxicity indicating its contribution to the adaptation of plants to stress. An increase in the lignin content mediated by Zn toxicity has also been observed in *Thlaspi caerulescens* J.Presl & C.Presl, following the upregulation of the related biosynthetic genes (Van De Mortel et al., 2006). Interestingly, the lignin deposition under Zn toxicity was higher in *Thlaspi caerulescens* compared with its glycophyte relative, *Arabidopsis thaliana* with higher sensitivity to Zn toxicity, indicating again a relationship between the higher ability to lignin synthesis and tolerance to not only salt (Hajiboland et al., 2020) but also to Zn toxicity (Van De Mortel et al., 2006).

4.5 THE EFFECT OF SINGLE AND COMBINATIVE TREATMENTS OF SALT AND ZN TOXICITY ON THE ACCUMULATION OF ELEMENTS

It has been observed that salt treatment results in higher accumulation of Zn in *B. juncea* (Novo et al., 2014) and *Brassica rapa* L. (Zeiner et al., 2022) because of salt-mediated increase in the mobility of Zn in the soil and within plants and enhancement of the root-shoot translocation of Zn in these glycophyte species (Novo et al., 2014). In the present work, the application of salt reduced Zn accumulation in the aerial parts of *L. latifolium*. Although there is no information about other halophytes of this family, similar results have been obtained for Cd in the halophytes from Aizoaceae (*Carpobrotus rossii* (Haw.) Schwantes) in that application of salt decreased Cd concentration in plant's aerial parts (Cheng et al., 2018).

A relatively low concentration of Na in the leaves (6-18 mg g⁻¹ DM) was in agreement with the halophytic behavior of *L. latifolium*. It has been reported that under relatively low salt concentrations (50-150 mM), halophytes are more successful in controlling the Na uptake than glycophytes, and thus, do not accumulate much Na

under these conditions (Munns, 2005). Salt treatment significantly reduced the concentration of K and Ca similar to that observed in other halophytes, e.g. *Plantago coronopus* L. (Koyro, 2006).

Zn toxicity treatment increased the leaf Na concentration in salt-treated plants by about 2.3 fold. A similar increase was observed in K concentration however, it was a 'concentration effect' because the K content (mg plant⁻¹) was rather decreased by Zn treatment (data not shown). In *Kosteletzkya virginica* (L.) C.Presl ex A.Gray Zn toxicity increased Na and decreased K concentration in the aerial parts (Han et al., 2012). Damages to membranes under Zn toxicity are likely the mechanism for an increase in the Na concentration in Zn-stressed plants that may be in turn prevented the mitigating effect of the combinative treatment on the biomass in our plants.

5 CONCLUSIONS

In contrast to available reports on the ameliorative effects of salt on heavy metal tolerance in the halophytes, in this study, a growth improvement was not observed under the combination of salt and Zn toxicity compared with excess Zn as single stress. This may suggest that the mitigating effect of salt on heavy metal stress is related to the heavy metal and/or the halophyte species. Nevertheless, the improvement of leaf pigments and, reduction of MDA and Zn accumulation in the leaves under the combination of both treatments showed that salt treatment is still able to stimulate the defense mechanisms in plants for protecting membranes and photosynthetic pigments against excess Zn concentrations.

Lepidium latifolium plants grow under diverse environmental conditions, have a high growth rate, and produce considerable biomass with perennial habit. These properties make this species a suitable candidate for revegetation and rehabilitation of saline soils contaminated with heavy metals.

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7 REFERENCES

- Acosta-Motos, J.R., Ortuño, M.F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M.J., & Hernandez, J.A. (2017). Plant responses to salt stress: adaptive mechanisms. *Agronomy*, 7, 18. <https://doi.org/10.3390/agronomy7010018>
- Aghaleh, M., Niknam, V., Ebrahimzadeh, H., & Razavi, K. (2009). Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biologia Plantarum*, 53, 243–248. <https://doi.org/10.1007/s10535-009-0046-7>
- Ali, M.B., Singh, N., Shohael, A.M., Hahn, E.J., & Paek, K.Y. (2006). Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress. *Plant Science*, 171, 147–154. <https://doi.org/10.1016/j.plantsci.2006.03.005>
- Arbelet-Bonnin, D., Ben-Hamed-Louati, I., Laurenti, P., Abdelly, C., Ben-Hamed, K., & Bouteaum F. (2019). *Cakile maritima* is a promising model for halophyte studies and a putative cash crop for saline agriculture. *Advances in Agronomy*, 155, 45–78. <https://doi.org/10.1016/bs.agron.2019.01.003>
- Arvouet-Grand, A., Vennat, B., Pourrat, A., & Legret, P. (1994). Standardization of propolis extract and identification of principal constituents. *Journal de Pharmacie de Belgique*, 49, 462–468.
- Ashraf, M.P., & Harris, P.J. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166, 3–16. <https://doi.org/10.1016/j.plantsci.2003.10.024>
- Balaferj, H., Bogusz, D., Triqui, Z.E., Guedira, A., Bendaou, N., Smouni, A., & Fahr, M. (2020). Zinc hyperaccumulation in plants: A review. *Plants*, 9, 562. <https://doi.org/10.3390/plants9050562>
- Bankaji, I., Sleimi, N., Gómez-Cadenas, A., & Pérez-Clemente, R.M. (2016). NaCl protects against Cd and Cu-induced toxicity in the halophyte *Atriplex halimus*. *Spanish Journal of Agricultural Research*, 14, e0810. <http://dx.doi.org/10.5424/sjar/2016144-10117>
- Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39, 205–207. <https://doi.org/10.1007/BF00018060>
- Ben Amor, N., Jiménez, A., Boudabbous, M., Sevilla, F., & Abdelly, C. (2020). Chloroplast Implication in the tolerance to salinity of the halophyte *Cakile maritima*. *Russian Journal of Plant Physiology*, 67, 507–514. <https://doi.org/10.1134/S1021443720030048>
- Boominathan, R., & Doran, P.M. (2002). Ni-induced oxidative stress in roots of the Ni hyperaccumulator, *Alyssum bertolonii*. *New Phytologist*, 156, 205–215. <https://doi.org/10.1046/j.1469-8137.2002.00506.x>
- Boughalleb, F., & Denden, M. (2011). Physiological and biochemical changes of two halophytes, *Nitraria retusa* (Forssk.) and *Atriplex halimus* (L.) under increasing salinity. *Agriculture Journal*, 6, 327–339.
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cambrollé, J., Mancilla-Leytón, J.M., Muñoz-Vallés, S., Luque, T., & Figueroa, M.E. (2012). Zinc tolerance and accumulation in the salt-marsh shrub *Halimione portulacoides*. *Chemosphere*, 86, 867–874. <https://doi.org/10.1016/j.chemosphere.2011.10.039>
- Casado-Vela, J., Sellés, S., & Bru, R. (2005). Purification and kinetic characterization of polyphenol oxidase from tomato fruits (*Lycopersicon esculentum* cv. Muchamiel). *Journal of Food Biochemistry*, 29, 381–401. <https://doi.org/10.1111/j.1745-4514.2005.00037.x>
- Černý, M., Habánová, H., Berka, M., Luklová, M., & Brzobohatý, B. (2018). Hydrogen peroxide: its role in plant biology and crosstalk with signalling networks. *International Journal of Molecular Sciences*, 19, 2812. <https://doi.org/10.3390/ijms19092812>
- Chance, B., & Maehly, A.C. (1954). Assay of catalases and peroxidases. *Methods of Biochemical Analysis*, 1, 357–424. <https://doi.org/10.1002/9780470110171.ch14>
- Chen, S., Wang, Q., Lu, H., Li, J., Yang, D., Liu, J., & Yan, C. (2019). Phenolic metabolism and related heavy metal tolerance mechanism in *Kandelia obovata* under Cd and Zn stress. *Ecotoxicology and Environmental Safety*, 169, 134–143. <https://doi.org/10.1016/j.ecoenv.2018.11.004>
- Cheng, M., Wang, A., Liu, Z., Gendall, A.R., Rochfort, S., & Tang, C. (2018). Sodium chloride decreases cadmium accumulation and changes the response of metabolites to cadmium stress in the halophyte *Carpobrotus rossii*. *Annals of Botany*, 122, 373–385. <https://doi.org/10.1093/aob/mcy077>
- Cheng, S., Yang, Z., Wang, M., Song, J., Sui, N., & Fan, H. (2014). Salinity improves chilling resistance in *Suaeda salsa*. *Acta Physiologia Plantarum*, 36, 1823–1830. <https://doi.org/10.1007/s11738-014-1555-3>
- Chun, H.J., Baek, D., Cho, H.M., Lee, S.H., Jin, B.J., Yun, D.J., ... Kim, M.C. (2019). Lignin biosynthesis genes play critical roles in the adaptation of Arabidopsis plants to high-salt stress. *Plant Signaling & Behavior*, 14, 1625697. <https://doi.org/10.1080/15592324.2019.1625697>
- Dal Corso, G. (2012). Heavy metal toxicity in plants. In: Furini, A. (Eds.), *Plants and heavy metals* (pp. 1–25). Dordrecht, Netherlands: Springer. https://doi.org/10.1007/978-94-007-4441-7_1
- Debez, A., Ben Hamed, K., Grignon, C., & Abdelly, C. (2004). Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. *Plant and Soil*, 262, 179–189. <https://doi.org/10.1023/B:PLSO.0000037034.47247.67>
- Dickerson, D.P., Pascholati, S.F., Hagerman, A.E., Butlerm L.G., & Nicholson. R.L. (1984). Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiological Plant Pathology*, 25, 111–123. [https://doi.org/10.1016/0048-4059%20\(84\)90050-X](https://doi.org/10.1016/0048-4059%20(84)90050-X)
- Ellouzi, H., Ben Hamed, K., Cela, J., Munné-Bosch, S., & Abdelly, C. (2011). Early effects of salt stress on the physiological and oxidative status of *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte). *Physiologia Plantarum*, 142, 128–143. <https://doi.org/10.1111/j.1399-3054.2011.01450.x>
- Fedenko, V.S., Landi, M., & Shemet, S.A. (2022). Metallophenolics: a novel integrated approach to study complexation of plant phenolics with metal/metalloid ions. *Internationa*

- tional Journal of Molecular Sciences*, 23, 11370. <https://doi.org/10.3390/ijms231911370>
- Francis, A., & Warwick, S.I. (2007). The biology of invasive alien plants in Canada. 8. *Lepidium latifolium* L. *Canadian Journal of Plant Science*, 87, 639–658. <https://doi.org/10.4141/CJPS06044>
- Gall, H.L., Philippe, F., Domon, J.M., Gillet, F., Pelloux, J., & Rayon, C. (2015). Cell wall metabolism in response to abiotic stress. *Plants*, 4:112–166. <https://doi.org/10.3390/plants4010112>
- Gao, D., Wang, Q., Wu, Y., Xu, H., Yu, Q., & Liu, J. (2008). Microsatellite DNA loci from the typical halophyte *Thellungiella salsuginea* (Brassicaceae). *Conservation Genetics*, 9, 953–955. <https://doi.org/10.1007/s10592-007-9403-2>
- Ghanem, A.M.F., Mohamed, E., Kasem, A.M., & 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>
- Ghnaya, T., Slama, I., Messedi, D., Grignon, C., Ghorbel, M.H., & Abdely, C. (2007). Effects of Cd²⁺ on K⁺, Ca²⁺ and N uptake in two halophytes *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*: consequences on growth. *Chemosphere*, 67, 72–79. <https://doi.org/10.1016/j.chemosphere.2006.09.064>
- Ghori, N.H., Ghori, T., Hayat, M.Q., Imadi, S.R., Gul, A., Altay, V., & Ozturk, M. (2019). Heavy metal stress and responses in plants. *International Journal of Environmental Science and Technology*, 16, 1807–1828. <https://doi.org/10.1007/s13762-019-02215-8>
- Giannopolitis, C.N., & Ries, S.K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, 59, 309–314. <https://doi.org/10.1104/pp.59.2.309>
- Guarino, F., Ruiz, K.B., Castiglione, S., Ciatelli, A., & Biondi, S. (2020). The combined effect of Cr (III) and NaCl determines changes in metal uptake, nutrient content, and gene expression in quinoa (*Chenopodium quinoa* Willd.). *Ecotoxicology and Environmental Safety*, 193, 110345. <https://doi.org/10.1016/j.ecoenv.2020.110345>
- Hafeez, B.M.K.Y., Khanif, Y.M., & Saleem, M. (2013). Role of zinc in plant nutrition—a review. *American Journal of Experimental Agriculture*, 3, 374. <https://doi.org/10.9734/AJEA/2013/2746>
- Hajiboland, R., Bahrami-Rad, S., Akhiani, H., & Poschenrieder, C. (2018). Salt tolerance mechanisms in three Irano-Turanian Brassicaceae halophytes relatives of *Arabidopsis thaliana*. *Journal of Plant Research*, 131, 1029–1046. <https://doi.org/10.1007/s10265-018-1053-6>
- Hajiboland, R., Bahrami-Rad, S., Zeinalzade, N., Atazadeh, E., Akhiani, H., & Poschenrieder, C. (2020). Differential functional traits underlying the contrasting salt tolerance in *Lepidium* species. *Plant and Soil*, 448, 315–334. <https://doi.org/10.1007/s11104-020-04436-0>
- Han, R.M., Lefèvre, I., Ruan, C.J., Qin, P., & Lutts, S. (2012). NaCl differently interferes with Cd and Zn toxicities in the wetland halophyte species *Kosteletzkya virginica* (L.) Presl. *Plant Growth Regulation* 68, 97–109. <https://doi.org/10.1007/s10725-012-9697-z>
- Han, X., Zhao, Y., Chen, Y., Xu, J., Jiang, C., Wang, X., Zhou, R., ... Zhang, J. (2022). Lignin biosynthesis and accumulation in response to abiotic stresses in woody plants. *Forestry Research*, 2, 9. <https://doi.org/10.48130/FR-2022-0009>
- Harinasut, P., Poonsopa, D., Roengmongkol, K., & Charoensataporn, R. (2003). Salinity effects on antioxidant enzymes in mulberry cultivar. *ScienceAsia*, 29, 109–113. <https://doi.org/10.2306/scienceasia1513-1874.2003.29.109>
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., & Ahmad A. (2012). Role of proline under changing environments: a review. *Plant Signaling & Behavior*, 7, 1456–1466. <https://doi.org/10.4161/psb.21949>
- Hodges, D.M., DeLong, J.M., Forney, C.F., & Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, 207, 604–611. <https://doi.org/10.1007/s004250050524>
- Kaur, T., Hussain, K., Koul, S., Vishwakarma, R., & Vyas, D. (2013). Evaluation of nutritional and antioxidant status of *Lepidium latifolium* Linn.: a novel phytofood from Ladakh. *PLoS ONE*, 8, e69112. <https://doi.org/10.1371/journal.pone.0069112>
- Keilig, K., & Ludwig-Müller, J. (2009). Effect of flavonoids on heavy metal tolerance in *Arabidopsis thaliana* seedlings. *Botanical Studies*, 50, 311–318.
- Kholodova, V.P., Volkov, K.S., & Kuznetsov, V.V. (2005). Adaptation of the common ice plant to high copper and zinc concentrations and their potential using for phytoremediation. *Russian Journal of Plant Physiology*, 52, 748–757. <https://doi.org/10.1007/s11183-005-0111-9>
- Kováčik, J., Klejdus, B., & Bačkor, M. (2009). Phenolic metabolism of *Matricaria chamomilla* plants exposed to nickel. *Journal of Plant Physiology*, 166, 1460–1464. <https://doi.org/10.1016/j.jplph.2009.03.002>
- Koyro, H.W. (2006). Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). *Environmental and Experimental Botany*, 56, 136–146. <https://doi.org/10.1016/j.envexpbot.2005.02.001>
- Kumar, R., Mishra, R.K., Mishra, V., Qidwai, A., Pandey, A., Shukla, S.K., ... Dikshit, A. (2016). Detoxification and tolerance of heavy metals in plants. In: Ahmad, P. (Eds.) *Plant metal interaction* (pp. 335–359). Elsevier. <https://doi.org/10.1016/B978-0-12-803158-2.00013-8>
- Kumari, A., Goyal, V., & Sheokand, S. (2019). Oxidative stress and antioxidant defense under metal toxicity in halophytes. In: Hasanuzzaman, M., Nahar, K., Öztürk, M. (Eds.) *Ecophysiology, abiotic stress responses and utilization of halophytes*. Springer (pp. 115–155), Singapore. https://doi.org/10.1007/978-981-13-3762-8_6
- Küpper, H., & Andresen, E. (2016). Mechanisms of metal toxicity in plants. *Metallomics*, 8, 269–285. <https://doi.org/10.1039/C5MT00244C>
- Leiková, A., Giehl, R.F., Hartmann, A., Fargaiová, A., & von Wirén, N. (2017). Heavy metals induce iron deficiency responses at different hierarchic and regulatory levels. *Plant Physiology*, 174, 1648–1668. <https://doi.org/10.1104/pp.16.01916>
- Lichtenthaler, H.K., & Welburn, A. (1983). Determination of

- total carotenoids and chlorophylls a and b of leaf extract in different solvents. *Biochemical Society Transactions*, 603, 591–592. <https://doi.org/10.1042/bst0110591>
- Lokhande, V.H., & Suprasanna, P. (2012). Prospects of halophytes in understanding and managing abiotic stress tolerance. In: Ahmad, P. & Prasad, M. (Eds.) *Environmental adaptations and stress tolerance of plants in the era of climate change*. Springer (pp. 29–56), New York. https://doi.org/10.1007/978-1-4614-0815-4_2
- MacFarlane, G.R., & Burchett, M.D. (2002). Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove *Avicennia marina* (Forsk.) Vierh. *Marine Environmental Research*, 54, 65–84. [https://doi.org/10.1016/S0141-1136\(02\)00095-8](https://doi.org/10.1016/S0141-1136(02)00095-8)
- Mahon, S., & Carman, K.R. (2008). The influence of salinity on the uptake, distribution, and excretion of metals by the smooth cordgrass, *Spartina alterniflora* (Loisel.), grown in sediment contaminated by multiple metals. *Estuaries and Coasts*, 31, 1089–1097. <https://doi.org/10.1007/s12237-008-9087-y>
- Manara, A. (2012). Plant responses to heavy metal toxicity. In: Furini A (Eds.) *Plants and heavy metals*. Springer (pp. 27–53), Dordrecht. <https://doi.org/10.1007/978-94-007-4441-7-2>
- McDonald, M., Mila, I., & Scalbert, A. (1996). Precipitation of metal ions by plant polyphenols: optimal conditions and origin of precipitation. *Journal of Agricultural and Food Chemistry*, 44, 599–606. <https://doi.org/10.1021/jf950459q>
- Mejias, C.L., Musa, J.C., & Otero, J. (2013). Exploratory evaluation of retranslocation and bioconcentration of heavy metals in three species of mangrove at Las Cucharillas marsh, *Journal of Tropical Life Science*, 3, 14–22. <https://doi.org/10.11594/jtls.03.01.03>
- Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, 15, 523–530.
- Morrison, I.M. (1972). A semi-micromethod for the determination of lignin and its use in predicting the digestibility of forage crops. *Journal of Science and Food Agriculture*, 23, 455–463. <https://doi.org/10.1002/jsfa.2740230405>
- Moura, J.C., Bonine, C.A., de Oliveira Fernandes Viana, J., Dornelas, M.C., & Mazzafera, P. (2010). Abiotic and biotic stresses and changes in the lignin content and composition in plants. *Journal of Integrative Plant Biology*, 52, 360–376. <https://doi.org/10.1111/j.1744-7909.2010.00892.x>
- Mummenhoff, K., Polster, A., Mühlhausen, A., & Theißen, G. (2009). *Lepidium* as a model system for studying the evolution of fruit development in Brassicaceae. *Journal of Experimental Botany*, 60, 1503–1513. <https://doi.org/10.1093/jxb/ern304>
- Munns, R. (2005). Genes and salt tolerance: bringing them together. *New Phytologist*, 167, 645–663. <https://doi.org/10.1111/j.1469-8137.2005.01487.x>
- Novo, L.A., Covelo, E.F., & González, L. (2014). Effect of salinity on zinc uptake by *Brassica juncea*. *International Journal of Phytoremediation*, 16, 704–718. <https://doi.org/10.1080/15226514.2013.856844>
- Peng, G., Lan, W., & Pan, K. (2022). Mechanisms of metal tolerance in halophytes: A mini review. *Bulletin of Environmental Contamination and Toxicology*, 109, 671–683. <https://doi.org/10.1007/s00128-022-03487-6>
- Qiu, N., & Lu, C. (2003). Enhanced tolerance of photosynthesis against high temperature damage in salt-adapted halophyte *Atriplex centralasiatica* plants. *Plant, Cell & Environment*, 26, 1137–1145. <https://doi.org/10.1046/j.1365-3040.2003.01038.x>
- 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, 50. <https://doi.org/10.3389/fpls.2016.00050>
- Ranieri, A., Castagna, A., Baldan, B., & Soldatini, G.F. (2001). Iron deficiency differently affects peroxidase isoforms in sunflower. *Journal of Experimental Botany*, 52, 25–35. <https://doi.org/10.1093/jexbot/52.354.25>
- Riyazuddin, R., Nisha, N., Ejaz, B., Khan, M.I.R., Kumar, M., Ramteke, P.W., & Gupta, R. (2021). A comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules*, 12, 43. <https://doi.org/10.3390/biom12010043>
- Sako, K., Nguyen, H.M., & Seki, M. (2020). Advances in chemical priming to enhance abiotic stress tolerance in plants. *Plant & Cell Physiology*, 61, 1995–2003. <https://doi.org/10.1093/pcp/pcaa119>
- Samanta, A., Das, G., & Das, S.K. (2011). Roles of flavonoids in plants. *International Journal of Pharmaceutical Science*, 6, 12–35.
- Sekmen, A.H., Turkan, I., Tanyolac, Z.O., Ozfidan, C., & Dinc, A. (2012). Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblancoolata* Bark. *Environmental and Experimental Botany*, 77, 63–76. <https://doi.org/10.1016/j.envexpbot.2011.10.012>
- Shackira, A.M., & Puthur, J.T. (2019). Cd²⁺ influences metabolism and elemental distribution in roots of *Acanthus ilicifolius* L. *International Journal of Phytoremediation*, 21, 866–877. <https://doi.org/10.1080/15226514.2019.1577356>
- Shah, S.S., Mohammad, F.I.D.A., Shafi, M., Bakht, J., & Zhou, W. (2011). Effects of cadmium and salinity on growth and photosynthesis parameters of *Brassica* species. *Pakistan Journal of Botany*, 43(1), 333–340.
- Sharma, S.S., Dietz, K.J., & Mimura, T. (2016). Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. *Plant, Cell & Environment*, 39, 1112–1126. <https://doi.org/10.1111/pce.12706>
- Siddique, A., Kandpal, G., & Kumar, P. (2018). Proline accumulation and its defensive role under diverse stress condition in plants: An overview. *Journal of Pure and Applied Microbiology*, 12, 1655–1659. <https://doi.org/10.22207/JPAM.12.3.73>
- Spent, R.O.L. (2006). The biology and ecology of *Lepidium latifolium* L. in the San Francisco Estuary and their implications for eradication of this invasive weed. Dissertation, University of California, Davis.
- Sruthi, P., Shackira, A.M., & Puthur, J.T. (2017). Heavy metal detoxification mechanisms in halophytes: an overview. *Wetlands Ecology and Management*, 25, 129–148. <https://doi.org/10.1007/s11273-016-9513-z>

- Swain, T., & Hillis, E.E. (1959). The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. *Journal of Science and Food Agriculture*, *10*, 63–68. <https://doi.org/10.1002/jsfa.2740100110>
- Uarrota, V.G., Stefen, D.L.V., Leolato, L.S., Gindri, D.M., & Nerling, D. (2018). Revisiting carotenoids and their role in plant stress responses: from biosynthesis to plant signaling mechanisms during stress. In: Gupta, D., Palma, J., Corpas, F. (Eds.) *Antioxidants and antioxidant enzymes in higher plants*. Springer (pp. 207–232), Switzerland. https://doi.org/10.1007/978-3-319-75088-0_10
- Van De Mortel, J.E., Almar Villanueva, L., Schat, H., Kwekkeboom, J., Coughlan, S., Moerland, P.D., ... Aarts, M.G. (2006). Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology*, *142*, 1127–1147. <https://doi.org/10.1104/pp.106.082073>
- Van Oosten, M.J., & Maggio, A. (2015). Functional biology of halophytes in the phytoremediation of heavy metal contaminated soils. *Environmental and Experimental Botany*, *111*, 135–146. <https://doi.org/10.1016/j.envexpbot.2014.11.010>
- Van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annual Review of Plant Biology*, *71*, 403–433. <https://doi.org/10.1146/annurev-arplant-050718-100005>
- Veitch, N.C. (2004). Structural determinants of plant peroxidase function. *Phytochemistry Reviews*, *3*, 3–18. <https://doi.org/10.1023/B:PHYT.0000047799.17604.94>
- Viehweger, K. (2014). How plants cope with heavy metals. *Botanical Studies*, *55*, 1–12. <https://doi.org/10.1186/1999-3110-55-35>
- Wallace, G., & Fry, S.C. (1994). Phenolic components of the plant cell wall. *International Review of Cytology*, *151*, 229–267. [https://doi.org/10.1016/S0074-7696\(08\)62634-0](https://doi.org/10.1016/S0074-7696(08)62634-0)
- Yang, Y., Shi, R., Wei, X., Fan, Q., & An, L. (2010). Effect of salinity on antioxidant enzymes in calli of the halophyte *Nitraria tangutorum* Bobr. *Plant, Cell, Tissue & Organ Culture*, *102*, 387–395. <https://doi.org/10.1007/s11240-010-9745-1>
- Zeiner, M., Juranović-Cindrić, I., Nemet, I., Franjković, K., & Salopek-Sondi, B. (2022). Influence of soil salinity on selected element contents in different *Brassica* species. *Molecules*, *27*, 1878. <https://doi.org/10.3390/molecules27061878>
- Zhou, M.X., Renard, M.E., Quinet, M., & Lutts, S. (2019). Effect of NaCl on proline and glycinebetaine metabolism in *Kosteletzkya pentacarpos* exposed to Cd and Zn toxicities. *Plant and Soil*, *441*, 525–542. <https://doi.org/10.1007/s11104-019-04143-5>