

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF (*APTENIA CORDIFOLIA*) TO SALT STRESS AND ITS REMEDIATIVE EFFECT ON SALINE SOILS

KARAKAS, S.^{1*} – DIKILITAS, M.² – ALMACA, A.¹ – TIPIRDAMAZ, R.³

¹Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Harran University, Sanliurfa, Turkey

²Department of Plant Protection, Faculty of Agriculture, Harran University, Sanliurfa, Turkey

³Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey

*Corresponding author

e-mail: skarakas@harran.edu.tr; phone: +90-414-318-3679

(Received 9th Sep 2019; accepted 21st Jan 2020)

Abstract. Salinization is one of the most significant environmental problems in the world. Salinity negatively affects the physicochemical properties of the soil and reduces crop production. This study aimed to investigate the potential use of *Aptenia cordifolia* L. for the phytoremediation of salt-affected soils. Three salt levels; non-saline (NS, EC: 1.38 dS m⁻¹), slightly saline (SS, EC: 3.54 dS m⁻¹) and highly saline (HS, EC: 9.58 dS m⁻¹) soils were collected from Harran Plain-Turkey and used to cultivate *A. cordifolia* in pots. To assess the salt tolerance of the plants, physiological and biochemical parameters as well as the accumulation of leaf Na⁺ and Cl⁻ ions were determined. In the meantime, soils were evaluated in terms of electrical conductivity, pH, organic matter and soil enzymes (dehydrogenase, urease, phosphatase and protease) before and after the cultivation. A significant increase in shoot fresh weight and dry weight were obtained from *A. cordifolia* cultivated-SS soils. No significant differences were determined in terms of crop yield in NS and HS soils. A decrease in total chlorophyll content was evident only in plants growing in HS soils. Proline and malondialdehyde contents and the activity of catalase and peroxidase enzymes were found significantly higher ($P \leq 0.05$) in plants growing in HS as compared to those growing in SS and NS soils. *A. cordifolia* plants, accumulated more Na⁺ and Cl⁻ ions in their leaves as the salinity levels increased. Soil pH levels under all soil conditions were not affected although slight increases were observed. However, soil electrical conductivity were decreased 2 and 2.5 times in SS and HS soil types, respectively. Soil organic matter were significantly increased along with the increase in the activity of soil enzymes in all soil types. We suggest that cultivation *A. cordifolia* in SS and HS soils will improve the condition of soil physicochemical properties in an environmentally-friendly manner.

Keywords: abiotic stress, phytoremediation, soil enzymes, halophytes, ameliorate

Introduction

Salinization is a worldwide problem in which salts gradually accumulate in the soil. In this process, water-soluble salts in the soils are dissolved via irrigation or rain and transferred to upper part of the soil and can then be accumulated when irrigation or rain ceased in semi-arid or arid environments (Panta et al., 2018). This negatively affects the crop productivity, quality and microbial community thus affects soil health (FAO, 2016). Due to soil salinization, soil infertility has been in an increasing trend (Slama et al., 2015). If serious measurements have not been taken, salt stress not only negatively affects the physical and chemical properties of the soil but also reduces the concentration of microorganisms and decrease the soil fertility. On the other hand, salinity reduces the quality and quantity of crops and in severe cases, it would not allow any seeds to germinate in which the standing point of agriculture (Yuan et al., 2007;

Negrao et al., 2017). Salt stress also leads to the production of reactive oxygen species (ROS) in crop plants that disrupt the membrane functionality and damages protein, lipid and nucleic acids. Although plants are able to develop a series of ROS detoxification mechanisms to minimize the impact of ROS via antioxidative enzyme systems including catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APX) and non-enzymatic metabolites (ascorbate, proline, glutathione etc.), this may not be enough for plants to function under harsh conditions. Even resistant plants may not cope with the ongoing stress such as salinity and heavy metal stress (Gill and Tuteja, 2010; Hossain et al., 2017).

In nature, plants develop various physiological and biochemical mechanisms in order to survive in soils characterized with salinity. General salt tolerance mechanisms include ion homeostasis and compartmentalization, ion transport and uptake and synthesis of osmoprotectants via compatible solutes (Gupta and Huang, 2014). Halophytes are plants that can tolerate and grow in soils characterized with high salt concentration. They, in general, are known to accumulate salts in various plant parts such as leaf, root etc. (Grigore et al., 2014; Flowers and Colmer).

Various physical, chemical and biological techniques can be used to remediate salt contaminated soils. The use of halophytes is one of the most promising and cost-effective methods for phytoremediation. It is a strategy where plants are used to remove, immobilize, degrade the soil contaminants such as salt and heavy metals (Kaushal et al., 2015). Phytoremediation has been used to manage wastes, especially petroleum hydrocarbons, polycyclic aromatic hydrocarbon, organic matter, and nutrients. So far a number of plants have been identified in use of phytoremediation purposes. Reclamation of salt-affected soils could be achieved and crop plants could grow in less stressed soils. Glycophyte plants could be grown together with the halophytes as companion plants in such soils. Also, the harvested above-ground biomass of halophytes can be used as animal feed (Ammari et al., 2013; Karakas et al., 2013).

A. cordifolia (L. f.) Schwant, also known as *Mesembryanthemum cordifolium*, is a species of succulent plant from the Aizoaceae family and has a Crassulacean acid metabolism (CAM) (Herppich and Peckmann, 1997; Tripodi and Modesta, 2003). In this study, we aimed to determine the performance of *A. cordifolia* against salt stress and to evaluate its performance under saline conditions for phytoremediation purposes. We determined the shoot fresh and dry weight, total chlorophyll, proline, malondialdehyde (MDA), Na^+ and Cl^- ions levels and catalase (CAT, E.C.1.11.1.6) and peroxidase (POX, E.C.1.11.1.7) enzyme responses of *A. cordifolia*. We also elucidated the physical and chemical properties of the soil in the vicinity of root area of *A. cordifolia* to determine the remediation capacity of the halophyte through measurement of soil electrical conductivity (EC), pH, organic matter (OM), ion (Na^+ , Cl^-) contents and soil enzyme activities (dehydrogenase, urease and protease) prior to and following cultivation with *A. cordifolia*.

Materials and methods

Experimental design and plant growth

Treatments consisted of three different soil salinity levels; non-saline (NS, EC: 1.38 dS m^{-1}), slightly saline (SS, EC: 3.54 dS m^{-1}) and highly saline (HS, EC: 9.58 dS m^{-1}) soils collected from Harran Plain-Turkey. Trials were performed in a randomized block design with three replicates. Before trial, soil samples were carefully air-dried to

allow sieving with a 2-mm mesh sieve. Three-week-old *A. cordifolia* seedlings were planted into 8-L pots containing 6 kg air-dried soil from each category in greenhouse ($35\text{ }^{\circ}\text{C} \pm 1$) conditions. The seedlings were planted on (April 2016) at a rate of 6 plants per pot. Pots were irrigated with full field capacity. Plants were then harvested on (July 2016) 100 days after onset of treatments. For the plant analysis, the shoot fresh weight (FW) and shoot dry weight (DW) were determined following harvest (Fig. 1).

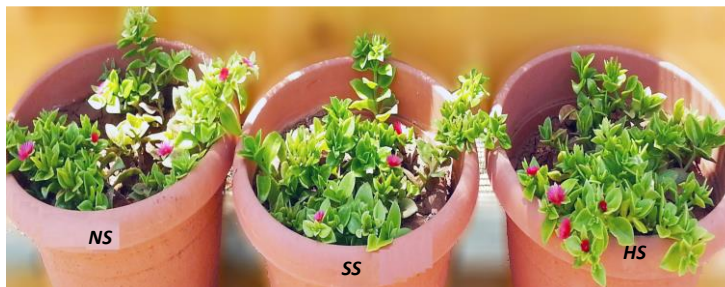


Figure 1. Growth of *A. cordifolia* at three different salinity levels

Total chlorophyll contents were determined according to the method of Arnon (1949) with slight modifications (Dikilitas, 2003). For the analysis, a 0.5 g leaf sample was homogenized in a 5 mL acetone: water (80:20% v/v) mixture. After filtration through Whatman No. 2 filter paper, a reading was made against 80% acetone blank for total chlorophyll at 652 nm using a UV spectrophotometer (UV-1700, Shimadzu). The results were then expressed as mg g^{-1} FW for total chlorophyll.

The proline measurement was conducted according to the method of Bates et al. (1973). Acid-ninhydrin was employed as a reagent, which was made by dissolving (warming and agitating) 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid. A quantity of 0.5 g of leaf material was homogenized in 10 mL of 3% w/v sulphosalicylic acid then the homogenate was filtered through Whatman No. 2 filter paper. A 2-mL of filtrate was mixed in a test tube with 2 mL of acid ninhydrin reagent and boiled at $100\text{ }^{\circ}\text{C}$ for 1 h. The reaction was terminated in an ice bath. The reaction mixture was extracted with 5 mL of toluene. Tubes were thoroughly shaken for 15-20 s and left for further 20 min in order to achieve separation of the two layers. The chromophore containing toluene was removed and allowed to warm to room temperature. Absorbance of the solution was measured at 515 nm using a toluene blank as a reference in a spectrophotometry (UV-1700, Shimadzu). The results were expressed as $\mu\text{mol g}^{-1}$ fresh tissue.

The malondialdehyde (MDA) content was determined according to the method of Sairam and Sexena (2003). A 0.5 g leaf tissue sample was homogenized using 10 mL of a 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at $10,000\text{ g}$ for 5 min. Four mL of 20% v/v TCA containing 0.5% v/v thiobarbituric acid (TBA) was added to 1 mL of the supernatant. The solution was heated at $95\text{ }^{\circ}\text{C}$ for 30 min and then quickly cooled on ice. The mixture was centrifuged once again at $10,000\text{ g}$ for 5 min and the absorbance of the clean supernatant was determined at 532 and 600 nm. The MDA content was calculated using Equation 1.

$$\text{MDA}(\text{nmol g}^{-1}) = \frac{\text{Extract volume (ml)} \times [(A_{532} - A_{600}) / (155 \text{ mM}^{-1} \text{ cm}^{-1})]}{\text{Sample amount (g)}} \times 10^3 \text{ (Eq.1)}$$

Catalase enzyme activity (CAT, E.C.1.11.1.6) was determined by monitoring the decomposition of H₂O₂ according to the method of Aebi (1984), with slight modifications (Karakas et al., 2016). For the analysis, 0.5 g of plant material was homogenized in 10 mL of a 50 mM Na-phosphate buffer solution then 50 µL of plant extract was added to a 2.95 mL (10 mM H₂O₂, 50 mM Na-phosphate buffer and 4 mM Na₂EDTA) reaction mixture and measured for 30 s at 240 nm with a UV spectrometer (UV-1700, Shimadzu). One CAT activity unit (U) is defined as a change of 0.1 absorbance unit per minute. Activity is expressed as enzyme units per gram fresh weight.

Peroxidase enzyme activity (POX, E.C.1.11.1.7) was determined by monitoring the increase in absorbance due to the tetraguaiacol formation at 470 nm according to the method of Cvikrova et al. (1994) with slight modifications (Karakas et al., 2016). For the analysis, 100 µL of extract (obtained as above) was added to 3 mL of the reaction mixture (13 mM guaiacol, 5 mM H₂O₂, and 50 mM Na-phosphate, pH 6.5). The reaction was initiated with an H₂O₂ addition and was measured at 470 nm using a UV spectrophotometer (UV-1700, Shimadzu) at 1-min interval until 3rd minute. One unit of POX activity is defined as a change of 0.1 absorbance unit per minute at 470 nm. Activity is expressed as enzyme units per gram of fresh weight.

The Na⁺ ion content of leaves was determined according to the method of Chapman and Pratt (1961). with slight modifications (Karakas et al., 2016). Samples burned at 500 °C were homogenized in 5 mL of a 2N HCl. For quantification of Na⁺ ions, the homogenate obtained following filtration was analyzed via Inductively Coupled Plasma (ICP, Perkin Elmer).

Chloride determination of plant samples was made according to the Mohr method using indicator (Johnson and Ulrich, 1959; Kacar and Inal, 2008).

Soil samples electrical conductivity (EC), pH, organic matter (OM) content and soil enzymes were determined before and after the trial. Soil EC and pH were determined using a saturated soil paste extraction (Soil Conservation Service, 1972; Thomas, 1996). OM content was determined according to Walkley (1947) and FAO (1974). One gram soil, 10 mL 1 N Cr₂O₇⁻ solution and 20 mL concentrated H₂SO₄ were poured into 500 mL beaker and mixed and allowed to stand for 30 min at room temperature. Then, 200 mL dH₂O and 10 mL concentrated orthophosphoric acid was added to the solution. The mixture was then cooled down to room temperature and 10-15 drops of diphenylamine indicator were added. The mixture was then titrated with 0.5 M FeH₈N₂O₈S₂ solution until the color changes from violet-blue to green. A reagent blank using the above procedure without soil was employed. The blank is used to standardize the Fe²⁺ solution daily. OM was calculated with *Equation 2*.

$$Total\ C(\%) = ((A - S) \times Fe^{+2} \times (0.003) \times (100 / B)) \quad (Eq.2)$$

To convert total C, the results were multiplied by 1.30 into the correction factor, OM, *Equation 3* was used.

$$OM(\%) = TotalC \times 1.72 / 0.58 \quad (Eq.3)$$

where A: blank titration ml ferrous solution, B: sample titration ml ferrous solution, 0.003: milliequivalent weight of C in g, factor of 1.72.

Dehydrogenase activity was measured using triphenyl tetrazolium chloride (TTC) colorimetric analysis according to Tabatabai (1994). The principle of dehydrogenase assay is that when metabolizing cells come in contact with an aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC) under anaerobic conditions, it is converted into triphenylformazan (TPF) and can be measured colorimetrically. Soil sample (5 g) was taken in 70 × 150 mm screw cap tubes. To each tube, 1 ml of 3% TTC and 5 ml of deionized water were added. Then the samples were mixed on a vortex, the tubes were stoppered and incubated in the dark for 24 h at 37 °C. After incubation, 10 ml of methanol were added, the samples were shaken and filtered Whatman filter paper No.1. After filtration, the optical reading was made at 485 nm with a UV spectrophotometer using methanol extract as the blank. The results were expressed as $\mu\text{mol TPF g}^{-1} \text{ soil h}^{-1}$.

The urease activity was determined following the method of Guan (1986) with some modifications. Briefly, 0.5 ml toluene, 20 ml of 0.1 M Na-citrate buffer (pH 6.7) and 10 ml of 10 % urea were added to 5.0 g soil and then the mixture was incubated at 37 °C for 24 h. One milliliter from the filtrate was obtained and added to 9 ml dH₂O and mixed with 4 ml Na-phenolate and 3 ml NaClO. The releasing ammonium was measured with a UV spectrophotometer at 578 nm. A control without urea was prepared with each sample. The results were expressed as $\text{mg urease-N kg}^{-1} \text{ h}^{-1}$.

Phosphatase activities were measured based on the colorimetric estimation of the *p*-nitrophenol release from *p*-nitrophenyl phosphate (Tabatabai, 1994). One gram soil was placed in a 50-mL Erlenmeyer flask and then 0.2 mL toluene, 0.1 M modified universal buffer (pH 6.5), 1 ml 0.1 M *p*-nitrophenyl phosphate (PNP) solution was added to the flask. The flask was swirled for a few seconds and incubated at 37 °C for 1 h in the incubator with a stopper. After removing the stopper, 1 ml of 0.5 M CaCl₂ and 4 ml of 0.5 M NaOH were added to the mixture. The soil suspension was filtered through Whatman No. 1 filter paper. The optical density of the filtrate was then measured at 420 nm in a spectrophotometer (UV-1700, Shimadzu). Blank was maintained similarly without soil. The results were expressed as $\text{mg P-nitrophenyl kg}^{-1} \text{ h}^{-1}$.

Protease activity was determined according to the method of Girard and Michaud (2002). Air-dried soil (0.1 g) was placed into 1.5 mL Eppendorf tube and then 500 μL of 1% (w/v) azocasein in 50 mmol L⁻¹ Tris-HCl, pH 8.8. Azocasein hydrolysis was initiated by incubating the tubes 2 h at room temperature. Proteolysis was stopped by adding 300 μL of 10 (w/v) cold trichloroacetic acid (TCA). After centrifugation for 10 min at 15000 g, 350 μL of the supernatant was collected and mixed with of 300 μL of 1 N NaOH. Protease activity was then determined by reading the optical density of the resulting solution at 440 nm using a UV spectrophotometer. A change of 0.01 units per minute in absorbance was considered to be equal to one unit protease activity, which was expressed as U (unit) $\text{mg}^{-1} \text{ protein}$.

Data were subjected to an analysis of variance (ANOVA) at a significance level of $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT) from the SPSS software program (Version 22.0). Data are presented as a mean value $\pm$ the standard error.

Results

Plant analysis of A. cordifolia under salinity levels

A. cordifolia plants were harvested after 100 days of cultivation from the soils differing in salinity levels. Growth of shoots was followed by measuring FW and DW

of the plants. At SS soil conditions, FW and DW of the plants significantly increased. At HS soil conditions, FW and DW of the plants did not significantly differ from those of control plants cultivated in NS soil conditions (Fig. 2A and B; Table 1).

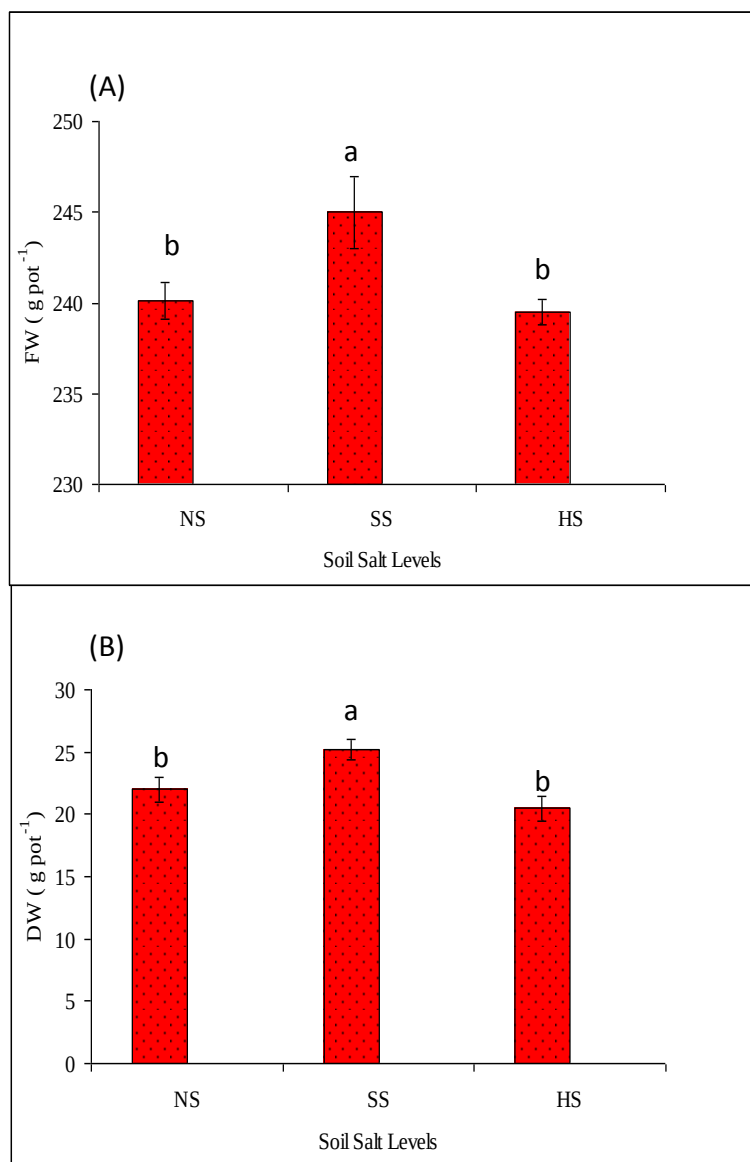


Figure 2. A) FW, B) DW of *A. cordifolia* plants at three different salinity levels: non-saline (NS), slightly saline (SS), and highly saline (HS) soils. Bars indicate the means of the three replicates $\pm$ standard error. Bars with different letters indicate significant differences from one another according to Duncan's Multiple Range Test at $P \leq 0.05$

When salinity levels increased, the accumulation of NaCl ions, Na⁺ and Cl⁻, in leaves of *A. cordifolia* also increased. The accumulation of both ions was more prominent of HS soil conditions. *A. cordifolia* plants accumulated approximately 16 g Na⁺ and 19 g Cl⁻ ions per kg leaf at SS soil conditions. On the other hand, *A. cordifolia* plants at HS soils accumulated almost 28 g kg⁻¹ and 32 g kg⁻¹ Na⁺ and Cl⁻ ions, respectively their leaves (Fig. 3A and B; Table 1).

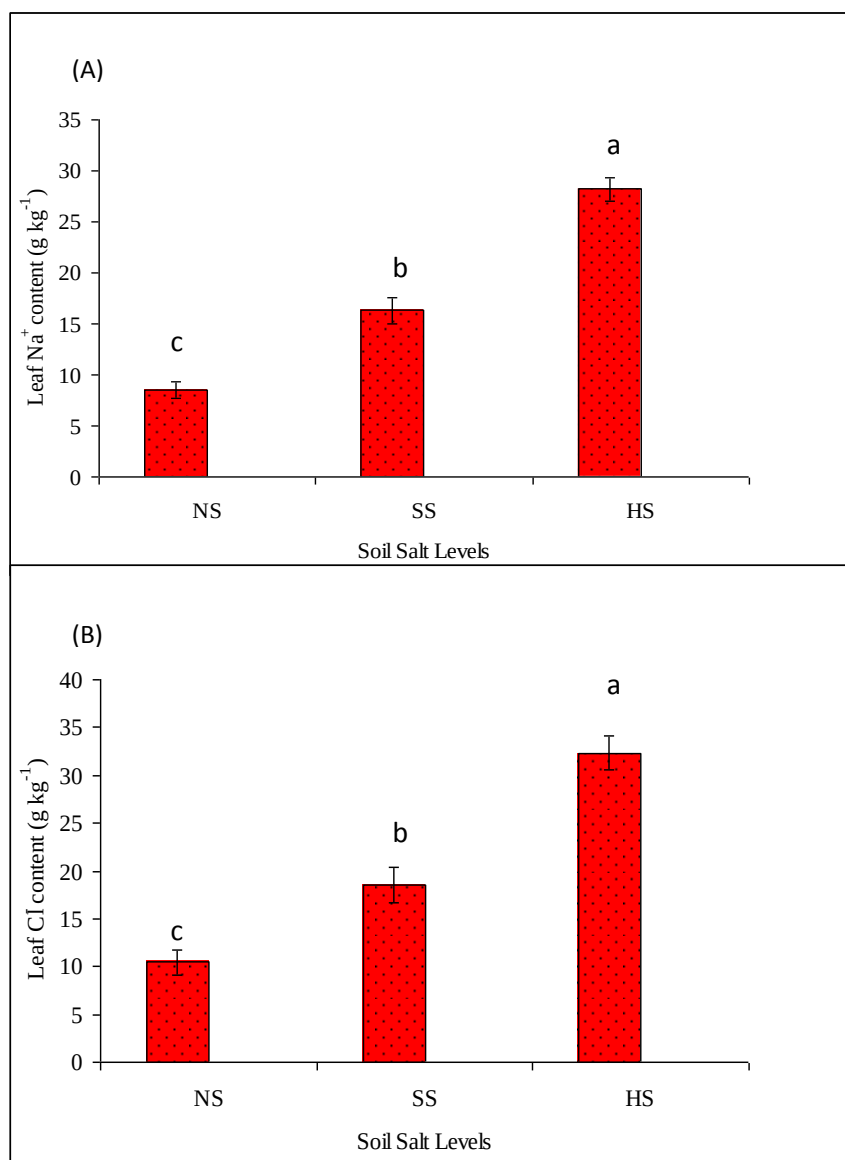


Figure 3. A) leaf Na⁺ content, B) leaf Cl⁻ content of *A. cordifolia* plants at three different salinity levels: non-saline (NS), slightly saline (SS), and highly saline (HS) soils. Bars indicate the means of the three replicates $\pm$ standard error. Bars with different letters indicate significant differences from one another according to Duncan's Multiple Range Test at $P \leq 0.05$

When biochemical parameters were examined under saline conditions, total chlorophyll contents were not statistically significant at all saline conditions (Fig. 3A). Both proline and MDA contents increased in plants subjected to HS soil conditions. The proline content was $1.9 \mu\text{mol g}^{-1}$ at HS conditions doubling the levels of NS and SS soil conditions. Again, MDA contents were at the highest levels at HS conditions (Fig. 4B and C).

As a response to salinity stress, both enzymes, POX and CAT, showed increasing trend as the concentration of NaCl increased in the soil. A significant increase was evident at HS soil conditions *A. cordifolia* plants did not exhibit significant enzyme responses at SS soil conditions (Fig. 4D and E; Table 1).

Table 1. Analyses of plant parameters using ANOVA

Plant parameters	Soil salt levels	n	Mean $\pm$ S.E	F	P
FW	NS	3	240.12 $\pm$ 1.15	9.07	0.02
	SS	3	245.11 $\pm$ 2.01		
	HS	3	239.43 $\pm$ 0.61		
	Total	9	241.55 $\pm$ 1.37		
DW	NS	3	21.99 $\pm$ 0.97	5.69	0.04
	SS	3	25.19 $\pm$ 0.87		
	HS	3	20.85 $\pm$ 0.98		
	Total	9	22.68 $\pm$ 0.80		
Leaf Na ⁺	NS	3	8.47 $\pm$ 0.55	86.20	0.00
	SS	3	16.33 $\pm$ 1.23		
	HS	3	28.20 $\pm$ 1.27		
	Total	9	17.67 $\pm$ 2.92		
Leaf Cl ⁻	NS	3	10.47 $\pm$ 1.35	41.41	0.00
	SS	3	18.54 $\pm$ 1.98		
	HS	3	32.35 $\pm$ 1.77		
	Total	9	20.46 $\pm$ 3.31		
Total chlorophyll	NS	3	1.51 $\pm$ 0.29	0.05	0.95
	SS	3	1.46 $\pm$ 0.14		
	HS	3	1.41 $\pm$ 0.28		
	Total	9	1.46 $\pm$ 0.12		
Proline	NS	3	0.96 $\pm$ 0.07	48.87	0.00
	SS	3	1.07 $\pm$ 0.09		
	HS	3	1.91 $\pm$ 0.06		
	Total	9	1.310.15		
MDA	NS	3	1.40 $\pm$ 0.21	49.40	0.00
	SS	3	1.71 $\pm$ 0.29		
	HS	3	5.25 $\pm$ 0.38		
	Total	9	2.79 $\pm$ 0.64		
CAT	NS	3	0.43 $\pm$ 0.08	6.09	0.04
	SS	3	0.52 $\pm$ 0.10		
	HS	3	0.96 $\pm$ 0.16		
	Total	9	0.64 $\pm$ 0.10		
POX	NS	3	2.41 $\pm$ 0.23	26.31	0.00
	SS	3	2.72 $\pm$ 0.25		
	HS	3	6.28 $\pm$ 0.64		
	Total	9	3.80 $\pm$ 0.65		

Soil analysis of *A. cordifolia* under salinity levels

Soil characteristics such as soil pH, EC and OM contents were evaluated bare soil before (control) and after cultivation with *A. cordifolia*. To make a comprehensive evaluation, bare soil (not cultivated with any plants) was also employed when pH, EC and OM contents were measured. In this trial, physical soil parameters were determined and recorded in all soil conditions before and after 100-days cultivation with *A.*

cordifolia. The same parameters were-remeasured to see the differences in measured soil parameters. According to our findings, soil pH levels measured at different salinity conditions did not change throughout the course of the experiment. Cultivation of saline soils with *A. cordifolia* plants increased soil pH levels, however, this was not significant as compared to those of soils with no-cultivation (Fig. 5A; Table 2).

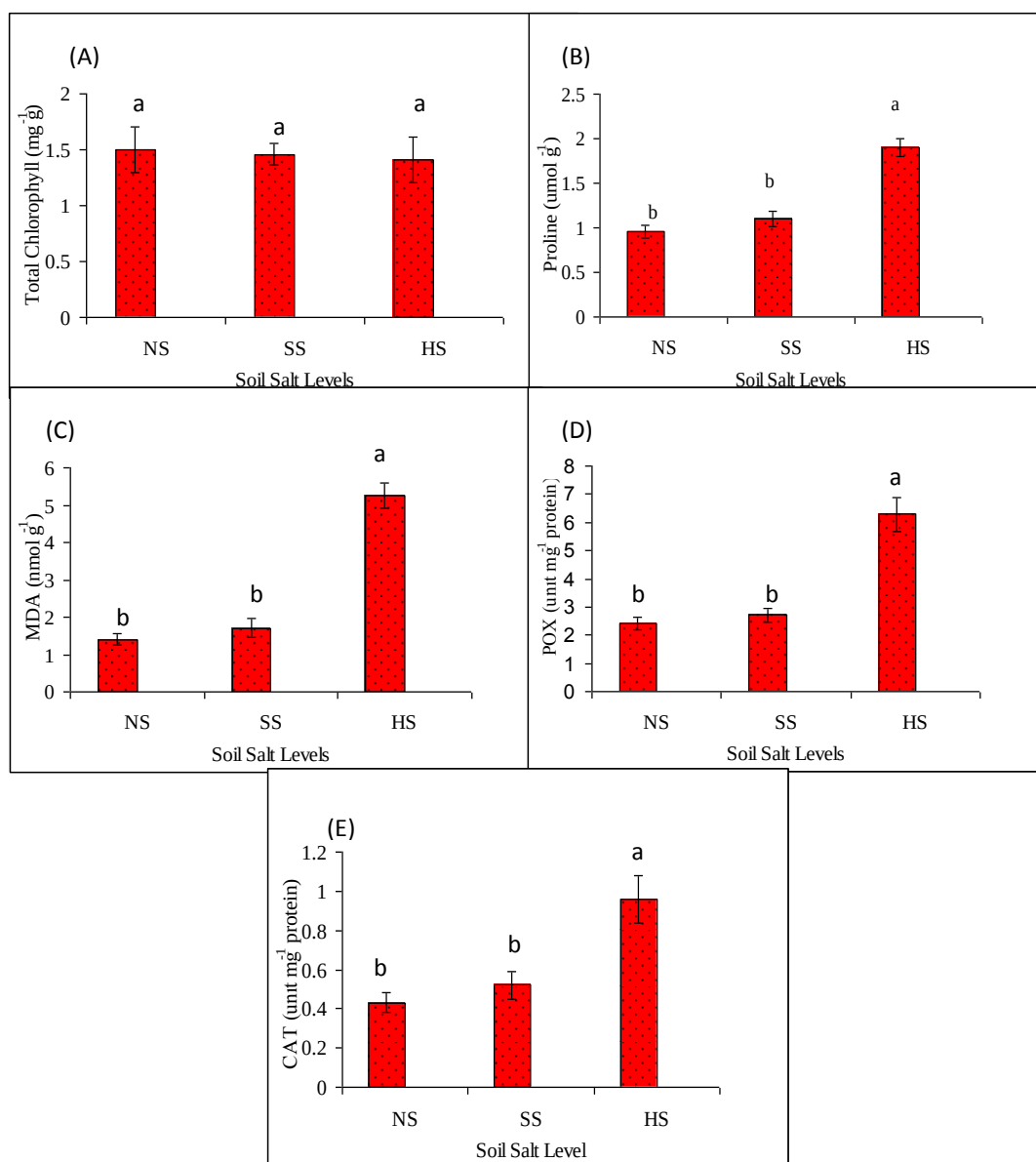


Figure 4. A) Total chlorophyll; B) proline; C) MDA; D) POX and E) CAT of *A. cordifolia* plants at three different salinity levels: non-saline (NS), slightly saline (SS), and highly saline (HS) soils. Bars indicate the means of the three replicates ± standard error. Bars with different letters indicate significant differences from one another according to Duncan's Multiple Range Test at $P \leq 0.05$

Use of *A. cordifolia* also affected the contents of OM in all soil conditions including SS soil conditions. Cultivation with *A. cordifolia* improved the soil OM significantly

meaning that cultivation with *A. cordifolia* contributed soil OM by secreting organic materials from their root systems (Fig. 5B; Table 2).

When soil EC levels were measured, it was determined that soil EC levels were significantly decreased in all conditions after 100-days cultivation with *A. cordifolia* (Fig. 5C; Table 2). Decrease in soil EC level was more remarkable in HS soil conditions.

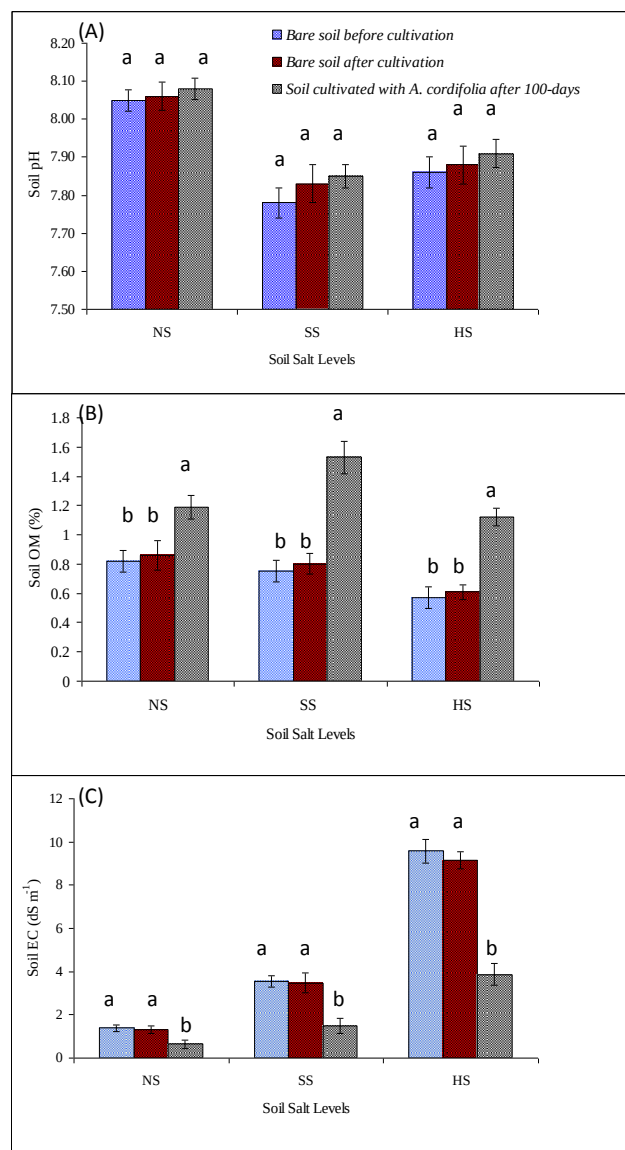


Figure 5. A) soil pH; B) soil OM; C) soil EC values of before and after cultivation with *A. cordifolia* at three different salinity levels: non-saline (NS), slightly saline (SS), and highly saline (HS) soils. Bars with different letters indicate significant differences from one another according to Duncan's Multiple Range Test at $P \leq 0.05$

Finally, soil enzymes were measured in all soil conditions to see if *A. cordifolia* plants contributed to soil health through increase in soil enzymes. Accordingly, cultivation with *A. cordifolia* significantly increased the soil enzymes such as dehydrogenase, urease, phosphatase and protease.

Table 2. Analyses of soil characteristics (pH, EC and OM) using ANOVA before and after cultivation with *A. cordifolia*

Soil salt levels	Application	pH				OM				EC			
		n	Mean ± S.E	F	P	n	Mean ± S.E	F	P	n	Mean ± S.E	F	P
NS	Bare soil before cultivated	3	8.05 ± 0.03	0.24	0.80	3	0.82 ± 0.07	5.76	0.04*	3	1.38 ± 0.15	6.82	0.02
	Bare soil after cultivated	3	8.06 ± 0.04			3	0.87 ± 0.10			3	1.34 ± 0.21		
	Soil cultivated with <i>A. cordifolia</i> after 100 days	3	8.08 ± 0.03			3	1.19 ± 0.08			3	0.65 ± 0.13		
	Total	9	8.06 ± 0.02			9	0.96 ± 0.07			9	1.12 ± 0.13		
SS	Bare soil before cultivated	3	7.78 ± 0.05	0.58	0.59	3	0.75 ± 0.10	16.43	0.00**	3	3.54 ± 0.35	7.29	0.03
	Bare soil after cultivated	3	7.83 ± 0.06			3	0.80 ± 0.08			3	3.46 ± 0.53		
	Soil cultivated with <i>A. cordifolia</i> after 100 days	3	7.85 ± 0.03			3	1.53 ± 0.14			3	1.48 ± 0.40		
	Total	9	7.82 ± 0.03			9	1.03 ± 0.14			9	2.83 ± 0.40		
HS	Bare soil before cultivated	3	7.86 ± 0.04	0.31	0.74	3	0.57 ± 0.15	9.88	0.01**	3	9.58 ± 0.64	34.41	0.00
	Bare soil after cultivated	3	7.88 ± 0.05			3	0.61 ± 0.06			3	9.14 ± 0.47		
	Soil cultivated with <i>A. cordifolia</i> after 100 days	3	7.91 ± 0.04			3	1.13 ± 0.05			3	3.87 ± 0.49		
	Total	9	7.88 ± 0.02			9	0.77 ± 0.10			9	7.53 ± 0.96		

A. cordifolia plants contributed to soil enzyme activities in NS soil conditions an indicating that soil health could be improved via cultivation with this plant (Figs. 6A, B, C and D; Table 3). Also, *A. cordifolia* plants improved soil enzyme levels in saline conditions. For example, soil dehydrogenase activity 1.5, soil urease activity 1.4, soil phosphatase activity 1.2 and soil protease activity 1.8 times increased after cultivation with *A. cordifolia* plants in HS soil conditions when compared to control soil (bare soil before cultivated). The increase in soil enzyme levels were more prominent in SS and HS soil conditions indicating that *A. cordifolia* plants played significant roles under saline stress conditions.

Table 3. Analyses of soil enzymes (dehydrogenase, urease, phosphatase, and protease) using ANOVA before and after cultivation with *A. cordifolia*

Soil salt levels	Application	Dehydrogenase				Urease				Phosphatase				Protease			
		n	Mean ± S.E.	F	P	n	Mean ± S.E.	F	P	n	Mean ± S.E.	F	P	n	Mean ± S.E.	F	P
NS	Bare soil before cultivated	3	19.10 ± 1.58	7.50	0.02	3	67.67 ± 0.88	11.45	0.01	3	142.33 ± 5.04	20.58	0.00	3	37.60 ± 3.75	5.03	0.05
	Bare soil after cultivated	3	20.10 ± 1.58			3	69.33 ± 1.45			3	150.00 ± 5.29			3	40.17 ± 0.73		
	Soil cultivated with <i>A. cordifolia</i> after 100 days	3	25.95 ± 0.69			3	78.10 ± 2.31			3	180.00 ± 2.08			3	48.50 ± 2.18		
	Total	9	21.72 ± 1.26			9	71.70 ± 1.82			9	157.44 ± 6.15			9	42.09 ± 2.08		
SS	Bare soil before cultivated	3	11.23 ± 1.40	17.70	0.00	3	54.73 ± 1.97	6.35	0.03	3	110.33 ± 3.76	24.35	0.00	3	26.10 ± 2.74	10.79	0.01
	Bare soil after cultivated	3	12.20 ± 1.01			3	58.46 ± 1.13			3	120.00 ± 7.64			3	27.00 ± 3.28		
	Soil cultivated with <i>A. cordifolia</i> after 100 days	3	19.28 ± 0.55			3	69.17 ± 4.62			3	160.00 ± 3.61			3	46.57 ± 4.35		
	Total	9	14.24 ± 1.37			9	60.79 ± 2.62			9	130.11 ± 8.06			9	33.22 ± 3.77		
HS	Bare soil before cultivated	3	4.28 ± 0.19	9.25	0.02	3	51.20 ± 1.61	10.99	0.01	3	81.00 ± 5.13	6.54	0.03	3	21.50 ± 1.59	7.06	0.03
	Bare soil after cultivated	3	4.07 ± 0.37			3	50.46 ± 1.96			3	85.00 ± 2.89			3	23.29 ± 1.09		
	Soil cultivated with <i>A. cordifolia</i> after 100 days	3	6.31 ± 0.57			3	68.90 ± 2.27			3	102.00 ± 4.73			3	37.54 ± 2.46		
	Total	9	4.89 ± 0.65			9	56.89 ± 2.12			9	89.33 ± 3.89			9	27.44 ± 1.65		

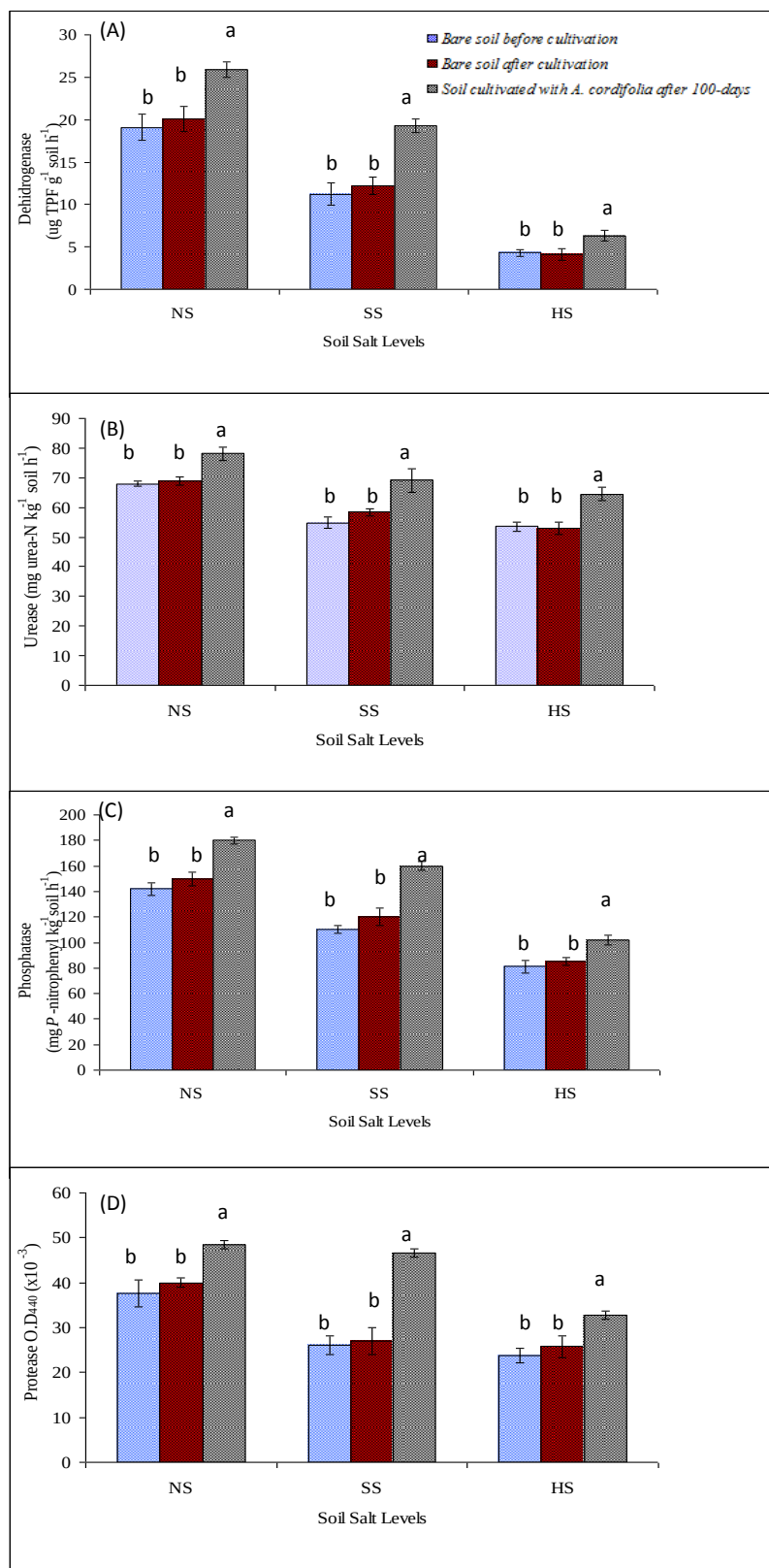


Figure 6. A) The soil dehydrogenase; B) urease; C) phosphatase; and D) protease values before and after cultivation with *A. cordifolia* at three different salinity levels: non-saline (NS), slightly saline (SS), and highly saline (HS) soils. Bars with different letters indicate significant differences from one another according to Duncan's Multiple Range Test at $P \leq 0.05$

Discussion

This study assessed the capacity of *A. cordifolia* to withstand salt stress. Phytoremediation and production potentials (biomass production and ion uptake) of *A. cordifolia* was evaluated at three different salinity levels in a 100-day pot experiment under controlled greenhouse conditions. Measurement was made in terms of growth, total chlorophyll, proline, malondialdehyde (MDA), mineral content, CAT and POX antioxidant enzyme changes as well as with the determination of soil EC, pH, OM, ion (Na^+ , Cl^-) contents and soil enzyme activities (dehydrogenase, urease, phosphatase and protease) before and after cultivation of such soils with *A. cordifolia*.

The results of this study showed that *A. cordifolia* plant was tolerant to both slight and high salinity. Accumulation of Na^+ and Cl^- ions increased in the leaves at both salinity levels. Therefore, this plant would be promising in reducing the electrical conductivity (EC) of such soils. The plants displayed great tolerance to the deleterious influence of salinity by preserving the integrity of their cell membranes by their proline and enzyme levels. During the growth period, proline contents and enzyme activities increased in the leaves under increasing saline levels. The accumulation of organic osmolytes such as proline, glycine betaine, sugar, alcohols, polyamines and proteins plays a key role in maintaining the low intracellular osmotic potential of plants and in preventing the harmful effects of salinity stress (Hýskova and Ryslava, 2018; Dutta et al., 2018). In our study, lipid peroxidation (MDA), POX and CAT activities increased significantly in *A. cordifolia* under salt stress. Salt stress, in general, increases lipid peroxidation and induces oxidative stress in plant tissues (Kim et al., 2016; Taibi et al., 2016). However, antioxidants such as POX and CAT tend to increase in tolerant cultivars, which scavenge H_2O_2 and other reactive oxygen species (ROS). These enzymes play significant roles in preventing cellular damages. We noticed that *A. cordifolia* plants were able to increase their organic metabolites upon stress exposure. It is important to note that those parameters were measured after 100 days in saline conditions. At SS conditions, FW and DW of the plants significantly increased. It is possible that SS might exert hormonal activity on *A. cordifolia* plants. This might have increased the FW and DW contents of the plants. Similar findings were stated that by Cela and Munné-Bosch (2012) on *A. cordifolia* was able to adapt to salt stress by increasing fresh biomass production. Therefore, tolerance of this plant after relatively long time under saline conditions is quite remarkable when compared to short-time stress of glycophytes in saline conditions under salinity stress. A plant with high tolerance to salinity with high capacity to accumulate Na^+ and Cl^- ions in their leaf or other parts including fruits is a good candidate for phytoremediation purposes (Bhuiyan et al., 2017; Karakas et al., 2017). *A. cordifolia* also improved the conditions of saline soils without spoiling the physical and chemical properties of such soils. Soil enzyme levels significantly increased along with the increase of OM contents and decrease in EC levels without changing pH balance of such soils. In most cases, changes in physical and chemical properties result in changes in pH levels (Miltner et al., 2012; Shrivastava and Kumar, 2015; Yang et al., 2018).

Soil enzyme activity is closely related to the carbon inputs, crop growth stages, organic matter content, microbial community structure, soil ecology, physical and chemical properties, vegetation, fertility, soil pH, soil quality and abiotic soil environment (Jin et al., 2009; Rout et al., 2017). Microbial population and decomposition of organic matter result in increased activity of soil enzymes. For example, high urease activity was observed during the active growth of maize (Jin et al.,

2009). The present study suggests that *A. cordifolia* can be used for soil improvement in saline areas. *A. cordifolia* could remediate structure of soil where salinity is predicted to occur. Indeed, this species behaves similarly to those of halophytes that tolerate moderate and high doses of salt. The use of this species over halophytes has a great advantage since its vegetative life is longer than halophytes, therefore, it has significantly more time to improve soil conditions under saline threat. Its ecological competence via rapid colonization in soils and accumulation of toxic ions in over those of other species could be considered as a good remediative plant. Our next approach is to investigate other properties of this plant if it is to be used as a good companion plant with other glycophytes to improve the potential vegetative and fruiting performance under saline stress conditions. It is important to note that the performance of *A. cordifolia* plants under heavy metal polluted soils would increase the popularity and use of this plant.

Conclusions

Salinity is one of the most significant environmental problems in arid and semi-arid regions. One of the alternate ways is to use of arable lands efficiently and to include the marginal quality of areas or non-agricultural lands into agricultural areas. These areas should be remediated via physical, chemical and biological approaches. Phytoremediation, in this aspect, has many advantageous; its major advantages are the low cost and environmentally-friendly sites. In this respect use of one of salt-tolerant (halophyte) plants is one of the cost-effective methods. Although salt-tolerant plants are less affected than their non-salt tolerant counterparts to the effect of salinity, however, the generation of salt-tolerant plants is very difficult and not cost-effective as planned. Those plants might possess salt tolerance properties unlike exhibiting disease susceptibility, therefore, the generation of salt-tolerant plants might not be good solution as its own. However, this could be achieved via the use of halophyte plants if they are used for phytoremediation purposes. Phytoremediation process not only remediate the conditions of soil but also reduces the stress level on crop plants. This approach could be practiced in all soil types in differing soil salinity levels and in all plant species when co-cultivated. With this approach, much cheaper and faster improvements could be achieved. Instead of developing salt-tolerance mechanisms via expensive arrays directly on crop plants, use of phytoremediation in saline stressed soils would reduce the impact of salinity indirectly via much safer ways.

This study assessed salt tolerance capacity of the *A. cordifolia* in three salt levels (NS, SS and HS). Its adaptation mechanisms and performances were assessed in terms of plant growth, total chlorophyll, accumulation of osmolyte proline, MDA, and antioxidative system (POX, CAT). *A. cordifolia* significantly increased soil OM and soil enzymes (dehydrogenase, urease, phosphatase and protease). We suggest that cultivation of *A. cordifolia* in slightly and highly saline soils would improve the condition of soil physicochemical and soil enzymes properties in an environmentally-friendly manner. *A. cordifolia* could be used in phytoremediation studies.

Acknowledgments. This study was financially supported by Harran University Scientific Research Project (HUBAP) no: 14077. We thank Dr. Murat Dikilitas for the biochemical analyses.

REFERENCES

- [1] Aebi, H. (1984): Catalase in Švitro. – Methods in Enzymology 105: 121-126.
- [2] Ammari, T. G., Al-Hiary, S., Al-Dabbas, M. (2013): Reclamation of saline calcareous soils using vegetative bioremediation as a potential approach. – Archives of Agronomy and Soil Science 59: 367-375.
- [3] Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris* L. – Plant Physiology 24: 1-15.
- [4] Bates, L. S., Waldren, R. P., Teare, I. D. (1973): Rapid determination of free proline for water-stress studies. – Plant Soil 39: 205-207.
- [5] Bhuiyan, M. S. I., Raman, A., Hodgkins, D. S. (2017): Plants in remediating salinity-affected agricultural and landscapes. – Proceedings of the Indian National Science Academy 83: 51-66.
- [6] Cela, J., Munné-Bosch, S. (2012): Acclimation to high salinity in the invasive CAM plant *Aptenia cordifolia*. – Plant Ecology and Diversity 5(3): 403-410.
- [7] Chapman, H. D., Pratt, P. F. (1961): Methods of Analysis for Soils, Plants, and Waters. – Division of Agricultural Sciences, University of California, Berkeley.
- [8] Cvikrova, M., Hrubcova, M., Vagner, M., Machackova, I., Eder, J. (1994): Phenolic acids and peroxidase activity in Alfalfa (*Medicago sativa*) embryogenic cultures after ethephon treatment. – Plant Physiology 91(2): 226-233.
- [9] Dikilitas, M. (2003): Effect of Salinity and Its Interactions with *Verticillium Albo-Atrum* on the Disease Development in Tomato (*Lycopersicon Esculentum* Mill.) and Lucerne (*Medicago Sativa* & *M. Media*) Plants. – University of Wales, Swansea.
- [10] Dutta, T., Neelapu, N.R.R., Wani, S. H., Challa, S. (2018): Compatible Solute Engineering of Crop Plants for Improved Tolerance toward Abiotic Stresses. – In: Wani, S. H. (ed.) Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants. Elsevier, Amsterdam.
- [11] FAO (1974): The Euphrates Pilot Irrigation Project. Methods of Soil Analysis, Gadeb Soil Laboratory (A Laboratory Manual). – FAO, Rome.
- [12] FAO (2016): Food and Agriculture: Key to Achieving the 2030, Agenda for Sustainable Development. – Food and Agriculture Organization of the United Nations, Rome, 23, I5499, <http://www.fao.org/3/a-i5499e>.
- [13] Flowers, T. J., Colmer, T. D. (2015): Plant salt tolerance: adaptations in halophytes. – Annals of Botany 115: 327-331.
- [14] Gill, S. S., Tuteja, N. (2010): Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – Plant Physiology and Biochemistry 48: 909-930.
- [15] Girard, C., Michaud, D. (2002): Direct monitoring of extracellular protease activities in microbial cultures. – Analytical Biochemistry 308(2): 388-391.
- [16] Grigore, M. N., Ivanescu, L., Toma, C. (2014): Halophyte Definitions and Classifications. – In: Grigore, M. N., Ivanescu, L., Toma, C. (eds.) An Integrative Anatomical Study. Springer, New York.
- [17] Guan, S. Y. (1986): Soil Enzymes and Their Research Methods. – Agricultural Sciencetech Press, Beijing, pp. 273-339.
- [18] Gupta, B., Huang, B. (2014): Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. – International Journal of Genomics. <http://dx.doi.org/10.1155/2014/701596>.
- [19] Herppich, W. B., Peckmann, K. (1997): Responses of gas exchange, photosynthesis, nocturnal acid accumulation and water relations of *Aptenia cordifolia* to short-term drought and rewatering. – Journal of Plant Physiology 150: 467-474.
- [20] Hossain, M. D., Inafuku, M., Iwasaki, H., Taira, N., Mostofa, M. G., Oku, H. (2017): Differential enzymatic defense mechanisms in leaves and root of two true mangrove species under long-term salt stress. – Aquatic Botany 142: 32-40.

- [21] Hýskova, V., Ryslava, H. (2018): Hyperosmotic versus hypoosmotic stress in plants. – *Biochemistry and Analytical Biochemistry* 7(1): 170.
- [22] Jin, K., Sleutel, S., Buchan, D., De Neve, S., Cai, D., Gabriels, D., Jin, J. (2009): Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. – *Soil and Tillage Research* 104(1): 115-120.
- [23] Johnson, C. M., Ulrich, A. (1959): II. Analytical methods for use in plant analysis. – *Calif Agric Exp Stat Bull* 766.
- [24] Kacar, B., Inal, A. (2008): *Plant Analysis*. – Nobel Publication and Distribution, Ankara.
- [25] Karakas, S. (2013): Development of tomato growing in soil differing in salt levels and effects of companion plants on same physiological parameters and soil remediation. – PhD, University of Harran, Sanliurfa.
- [26] Karakas, S., Cullu, M. A., Kaya, C., Dikilitas, M. (2016): Halophytic companion plants improve growth and physiological parameters of tomato plants grown under salinity. – *Pakistan Journal of Botany* 48: 21-28.
- [27] Karakas, S., Cullu, M. A., Dikilitas, M. (2017): Comparison of two halophyte species (*Salsola soda* and *Portulaca oleracea*) for salt removal potential under different soil salinity conditions. – *Turkish Journal of Agriculture and Forestry* 41: 183-190.
- [28] Kaushal, J., Bhasin, S. K., and Bhardwaj, P. (2015): Phytoremediation: a review focusing on phytoremediation mechanisms. – *International Journal of Research* 5: 1-9.
- [29] Kim, J., Liu, Y., Zhang, X., Zhao, B., Childs, K. (2016): Analysis of salt-induced physiological and proline changes in 46 switchgrass (*Panicum virgatum*) lines indicates multiple responses modes. – *Plant Physiology Biochemistry* 105: 203-212.
- [30] Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M. (2012): SOM genesis: microbial biomass as a significant source. – *Biogeochemistry* 111: 41-55.
- [31] Negrao, S., Schmockel, S. M., Tester, M. (2017): Evaluating physiological responses of plants to salinity stress. – *Annals of Botany* 119: 1-11.
- [32] Panta, S., Doyle, R., Hardie, M., Lane, P., Flowers, T., Haros, G., Shabala, S. (2018): Can highly saline irrigation water improve sodicity and alkalinity in sodic clayey subsoils? – *Journal of Soils and Sediments*. DOI: 10.1007/s11368-018-1986-3.
- [33] Rout, P. P., Chandrasekaran, N., Padhan, D. (2017): Soil enzyme activity as influenced by seasonal rainfall and crop growth stages under long-term fertilization and intensive cropping with hybrid maize. – *International Journal of Plant and Soil Science*. 18(2): 1-9.
- [34] Sairam, R. K., Sexena, D. (2000): Oxidative stress and antioxidants in wheat genotypes: a possible mechanism of water stress tolerance. – *Journal of Agronomy and Crop Science* 184: 55-61.
- [35] Shrivastava, P., Kumar, R. (2015): Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. – *Saudi Journal of Biological Sciences* 22: 123-131.
- [36] Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., Savoure, A. (2015): Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. – *Annals of Botany* 115: 433-447.
- [37] Soil Conservation Service (1972): *Soil Survey Laboratory Methods and Procedures for Collecting Soil Samples*. – Soil Survey Invest Rep No. 1. US Gov Print Office, Washington, DC.
- [38] Tabatabai, M. A. (1994): Soil Enzymes. – In: Weaver, W., Angel, J. S., Bottomley, P. S. (eds.) *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties*. SSSA Book Series 5, Soil Science Society of America, Madison, WI.
- [39] Taibi, K., Taibi, F., Abderrahim, L. A., Ennajah, A., Belkhodja, M., Mulet, J. M. (2016): Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defense systems in *Phaseolus vulgaris* L. – *South African Journal of Botany* 105: 306-312.
- [40] Thomas, G. W. (1996): Soil pH and Soil Acidity. – In: Sparks, D. L. (ed.) *Methods of Soil Analysis: Part 3. Chemical Methods*. Soil Science Society of America, Madison, WI.

- [41] Tripodi, K. E. J., Modesta, F. E. (2003): Purification and characterization of an NAD-dependent malate dehydrogenase from leaves of the crassulacean acid metabolism plant *Aptenia cordifolia*. – *Plant Physiology and Biochemistry* 41: 97-105.
- [42] Walkley, A. (1947): A critical examination of a rapid method for determining organic carbon in soils: effects of variations in digestion conditions and of inorganic soil constituents. – *Soil Sciences* 37: 29-38.
- [43] Yang, M., Yang, D., Yu, X (2018): Soil microbial communities and enzyme activities in sea-buckthorn (*Hippophae rhamnoides*) plantation at different ages. – *PLoS One* 13(1).
- [44] Yuan, B. C., Li, Z. Z., Liu, H., Gao, M., Zhang, Y. Y. (2007): Microbial biomass and activity in salt-affected soils under arid conditions. – *Applied Soil Ecology* 35: 319-328.