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Comparison of two halophyte species (*Salsola soda* and *Portulaca oleracea*) for salt removal potential under different soil salinity conditions

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Abstract: Salt-induced land degradation has gradually increased in several major irrigation schemes within arid and semiarid regions. To maximize crop productivity under saline conditions, either salt tolerance crops should be cultivated or areas should be desalinated. One of the most promising and cost-effective ways to maximize crop productivity is to use salt tolerant plants to remove salt from the soil. For this study, four levels of saline soils were cultivated with the halophyte species *Salsola soda* L. and *Portulaca oleracea* L. in pots. The soils had the following salinity levels: 1) nonsaline soil (NSS, 0.9 dS m^{-1}), 2) slightly saline soil (SSS, 4.2 dS m^{-1}), 3) moderately saline soil (MSS, 7.2 dS m^{-1}), and 4) highly saline soil (HSS, 14.1 dS m^{-1}). To assess the salt tolerance capacity of the halophytes, physiological and biochemical parameters as well as the accumulation of leaf Na^+ and Cl^- ions in the halophytes were investigated. Soils were additionally evaluated for electrical conductivity, pH, and soil ion concentrations prior to planting and the following harvest. The fresh and dry weights of both halophytes increased with increasing salinity levels ($P \leq 0.05$). The proline contents of *S. soda* and *P. oleracea* were 3.1 and 4.6 times higher, respectively, than within the same species grown under control conditions. The malondialdehyde and membrane stability index values for *S. soda* were insignificant under all salt conditions. Only *P. oleracea* showed significantly higher membrane damage under HSS conditions. In a similar manner, the chlorophyll content of both halophytes was not impacted for all of the salinity levels. Na^+ and Cl^- concentrations significantly decreased in soils that were planted with both halophytes ($P \leq 0.05$). The impact of *S. soda* on the removal of Na^+ from HSS was significantly higher than that of *P. oleracea* and removed 151.4 mmol Na^+ pot^{-1} as compared to the removal of 61.2 mmol Na^+ pot^{-1} by *P. oleracea.*

Key words: Halophytes, salt stress, *Salsola soda*, phytoremediation, *Portulaca oleracea*

1. Introduction

Agriculture is the art and science of cultivating the soil, growing crops, and raising livestock. It includes the preparation of plant and animal products for people to use and their distribution to markets. Agriculture not only provides food and raw material but also employment opportunities to a very large proportion of the population (Sahin et al., 2002; Erturk et al., 2010; Cucci et al., 2016; Sorkheh and Khaleghi, 2016).

Salinity is one of the world's most serious environmental stressors because it affects crop growth and agricultural productivity (Jouyban, 2012; Muhammad et al., 2015). Water sources on earth contain 30 g of sodium chloride per liter and so the earth is often stated to be a salty planet (Munns, 2002; Foolad, 2004). Although soil salinity existed prior to the advent of agriculture, the salinity problem in soils is now increasing at a rate of 10% annually (Shrivastava et al., 2015). Researchers have estimated that more than 50% of the earth's arable land could be salinized by 2050 (Jamil et al., 2011; Hasanuzzaman et al., 2014; Menason et al., 2015). Therefore, soil salinity has the capacity to influence plant growth via high concentrations of toxic ions as well as negative water potential (Dikilitas and Karakas, 2012).

The equilibrating osmotic potential within plant cells by excluding salt requires a great amount of energy and eventually results in nutrient imbalances within plant systems (Munns and Tester, 2008; Rahnama et al., 2010, Carrow and Duncan, 2011). To reduce the negative impact of salinity on crop plants, a considerable amount of salt should be removed from the vicinity of crop plants. Although salt-tolerant crop plants have been cultivated in recent years, the use of halophyte plants that remove salt from the vicinity of roots of crop plants has more potential for alleviating saline soils in the future (Roy et al., 2014; Karakas et al., 2016).

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In nature, plants respond to salinity in different ways. Some plants tolerate salt (Munns and Gilliham, 2015; Karakas et al., 2016) while others accumulate or exclude salt (Yuan et al., 2016). As a result, plants that thrive under saline conditions became an option for the remediation of saline-affected soils. Halophytes, plants that survive under salt concentrations greater than or equal to that of seawater, accumulate toxic ions in their vacuoles, accumulate compatible solutes in their cytoplasm, and activate genes for salt tolerance that confer salt resistance (Gorham, 1995; Zahoor et al., 2012). Although several methods such as physical (deep ploughing), chemical, and biological approaches have been established for the remediation of saline soils, the most promising and cost-effective is the use of halophyte species for saline areas (Qadir et al., 2007; Panta et al., 2014; Karakas, 2015). The plant-based method is of great importance, especially in developing countries where chemical amendments are becoming more and more expensive (Kumar and Abrol, 1984; Ahmad et al., 1990; Hasanuzzaman et al., 2014).

For this study, we determined the physiological and biochemical [proline, malondialdehyde (MDA), membrane stability index (MSI), chlorophyll, and mineral content] responses of *S. soda* and *P. oleracea*. Accumulation of these chemicals is a good indication of cell response under stress (Hassan et al., 2016; Gupta and Huang, 2014). However, increased contents of them in cells could also be considered as osmoprotectant under stress conditions to remediate the negative effects of stress. We also determined soil EC, pH, and ion (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-}) content prior to and following cultivation with halophytes. These species were regarded as salt tolerant species and they were only found in semiarid areas (Mekki, 2016). Therefore, it is important to determine their salt accumulation capacity and potential for removal of toxic ions from saline soils with various levels of salt.

2. Materials and methods

2.1. Greenhouse experiment

The halophyte species *S. soda* L. and *P. oleracea* L. were cultivated in 8-L pots containing 6 kg of air-dried soil with differing salinity levels that were collected from various locations of the salt-affected land of Harran Plain (36°52′39″N 39°02′02″E) in Turkey. Soil samples were then obtained from the top part (10–15 cm) of the soil surface. Soils selected for the trials had the following EC levels: 1) nonsaline soil (NSS), EC = 0.9 dS m^{-1}; 2) slightly saline soil (SSS), EC = 4.2 dS m^{-1}; 3) moderately saline soil (MSS), EC = 7.2 dS m^{-1}; and 4) highly saline soil (HSS), EC = 14.1 dS m^{-1}. Prior to the trials, the soil samples were air-dried to allow sieving with a 4-mm mesh sieve. Trials were performed in a randomized block design with four replicates. For each species and throughout the experiment, 30 seeds were germinated per pot and irrigated with tap water at 45% of the water soil field capacity. After 100 days, the trials were completed with the harvest of plants.

For the physiological analysis, the shoot fresh weight (FW) was determined following harvest. The dry weight (DW) of plants was determined after drying samples at 70 °C until they reached a constant weight. Soil samples were also collected in order to determine EC, pH, and ion content.

The membrane stability index (MSI) was determined as described by Premchandra et al. (1990). Leaf samples were cut into small pieces (5 mm in length) and placed in test tubes containing 10 mL of dH_2O. The tubes were placed in a water bath at 40 °C and the initial conductivity of the medium (C_1) was measured after 30 min. The samples were then further incubated at 100 °C for 10 min in order to expel electrolytes and then cooled to 25 °C, after which a second conductivity measurement of the medium (C_2) was performed. The MSI was calculated using the following equation:

$$MSI\% = [(C_2 - C_1)/(C_2)] \times 100$$

Chlorophyll content was determined based on the method reported by Arnon (1949). For the analysis, a 0.5-g leaf sample was homogenized in a 5-mL acetone:water (80:20% v/v) mixture. A reading was obtained against an 80% acetone blank for chlorophyll *a* at 663.5 nm and for chlorophyll *b* at 645 nm, using a UV spectrophotometer (UV-1700, Shimadzu).

The proline measurement was conducted as described by Bates et al. (1973). Acid-ninhydrin was used as a reagent. The reagent 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. Half a gram of leaf material was homogenized in 10 mL of 3% w/v sulfosalicylic acid using a pestle. The homogenate was filtered through Whatman No. 2 filter paper. Then 2 mL of filtrate was mixed in a test tube with 2 mL of acid ninhydrin reagent and boiled at 100 °C for 1 h. The reaction was terminated in an ice bath. The reaction mixture was then extracted using 5 mL of toluene. The tubes were thoroughly shaken for 15–20 s and left for 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 was then measured by spectrophotometry (UV-1700, Shimadzu) at 515 nm using a toluene blank as a reference.

The malondialdehyde (MDA) content was determined according to the method given by Sairam and Saxena (2000) with slight modifications. A 0.5-g leaf tissue sample was homogenized using 5 mL of 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 10,000 × *g* for 5 min. Next 4 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 °C for 30 min

and then quickly cooled on ice. The mixture was centrifuged again at 10,000 × *g* for 5 min and the absorbance of the clean supernatant was determined at 532 and 600 nm. Here, the MDA content of leaves is expressed as nmol g^{-1} fresh tissue.

The Na^+ ion content of leaves was determined according to Chapman and Pratt (1961) with slight modifications. Samples ashed at 500 °C were homogenized in 5 mL of 2 N HCl. For quantification of Na^+ ions, the homogenate obtained following filtration was analyzed via inductively coupled plasma (ICP, PerkinElmer).

Chloride determinations of plant samples were obtained according to the Mohr method using K_2CrO_7 indicator (Johnson and Ulrich, 1959; Kacar and İnal, 2008).

2.2. Soil analyses

Prior to planting, representative composite samples from each soil type (NSS, SSS, MSS, and HSS) were prepared in order to determine the initial physical and chemical properties of the soils so that initial and final values could be compared. Composite soil samples were prepared at harvest after removing root residue from each pot. Each collected soil sample was air-dried and ground so that it passed through a 2-mm sieve. Soil EC and pH and the water-soluble fractions of soil Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, and SO_4^{2-} were determined using a saturated soil paste extraction (Soil Conservation Service, 1972; Thomas, 1996). Cations were determined by ICP (PerkinElmer). Anions were measured by Shimadzu (HCI-20A-Super A3) column conductivity with 100-µL injection volume. The measurement was performed with 0.1% error.

The concentration of Na^+ ions removed by harvested halophytes was calculated according to the equation given by Qadir et al. (2003):

$$S_{Na\text{-}removal} = [(S_{Na\text{-}conc})\ (S_{DW})/(10^3)]/MW_{Na},$$

where $S_{Na\text{-}removal}$ is Na^+ removal through harvest (mmol pot^{-1}), $S_{Na\text{-}conc}$ is the ion concentration in the harvested plant (mg kg^{-1}), S_{DW} is the plant dry weight (g pot^{-1}), and MW_{Na} is the molecular weight of Na^+.

2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA) at a significance level of $P \leq 0.05$ using Duncan's multiple range test (DMRT) from SPSS (Version 11.0). The data are presented as mean values ± standard error.

3. Results and discussion

3.1. Plant parameters

The FW and DW of the halophytes were significantly greater for the MSS and HSS soil types than for SSS and NSS soil types. *S. soda* produced 43 g DW per pot while *P. oleracea* produced 40 g DW per pot in the HSS soil type after 100 days of cultivation. The halophytes produced almost twice as much DW compared to NSS treatment (Figures 1A and 1B).

The accumulation of Na^+ and Cl^- ions increased in the leaves of the two species as the salinity level increased. The Na^+ contents within the leaves of *S. soda* were 70.4 and 81.0 g kg^{-1} at MSS and HSS, respectively. On the other hand, the Na^+ contents within the leaves of *P. oleracea* were 25.8 and 35.2 g kg^{-1} at MSS and HSS, respectively. *S. soda* also accumulated Cl ions as 81.0 and 85.5 g kg^{-1} at MSS and HSS, respectively while *P. oleracea* accumulated Cl ions as 58.5 and 77.0 g kg^{-1} at MSS and HSS, respectively (Figures 1C and 1D).

When the salinity level was raised above SSS, the proline content increased in both *S. soda* and *P. oleracea*. Maximum proline content was observed for *S. soda* and *P. oleracea* under MSS and HSS conditions ($P \leq 0.05$) (Figure 2A). Nonsaline and slightly saline soil conditions did not cause significant increases in the proline content of the halophytes.

MDA was used as an indicator of membrane lipid peroxidation. However, increases in salinity did not cause changes in the MSI levels in *S. soda* and *P. oleracea*. MDA only increased in *P. oleracea* (Figures 2B and 2C).

The content of total chlorophyll in both halophytes was not statistically significant as the level of salinity increased (Figure 2D), indicating that the halophytes tolerated the negative influence of salt. Again, *S. soda* tolerated the deleterious impact of salt better than did *P. oleracea* (Figure 2D).

3.2. Soil parameters

We determined soil pH and EC values prior to and following the growth of halophytes. Soil EC drastically decreased following the growth of the two halophytes under the SSS, MSS, and HSS salinity levels. The EC of HSS was 3.27 dS m^{-1} and 5.16 dS m^{-1} following planting for *S. soda* and *P. oleracea,* respectively, as compared to 14.1 dS m^{-1} for nonplanted HSS (Figure 3A).

Results from our soil analysis, with respect to pH, indicated that the cultivation of *S. soda* and *P. oleracea* in saline soils did not affect the pH values of soil (Figure 3B).

Prior to planting (control), the values of soil soluble Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-} ions were higher within salt-affected soils. Increases in salinity levels were reflected in the ion concentrations. However, a reduction in ions was evident in all of the saline soils following halophyte planting. Although reductions in salt ions were achieved for both halophytes, *S. soda* contributed more to the reduction in ions as compared to *P. oleracea.* For example, the removal of Na^+ ions by *S. soda* was 1.9 times that of *P. oleracea* and similar to the removal of Cl^- ions, with *S. soda* being 1.7 times that of *P. oleracea.* As indicated in Figure 4, similar results were also obtained for other ions.

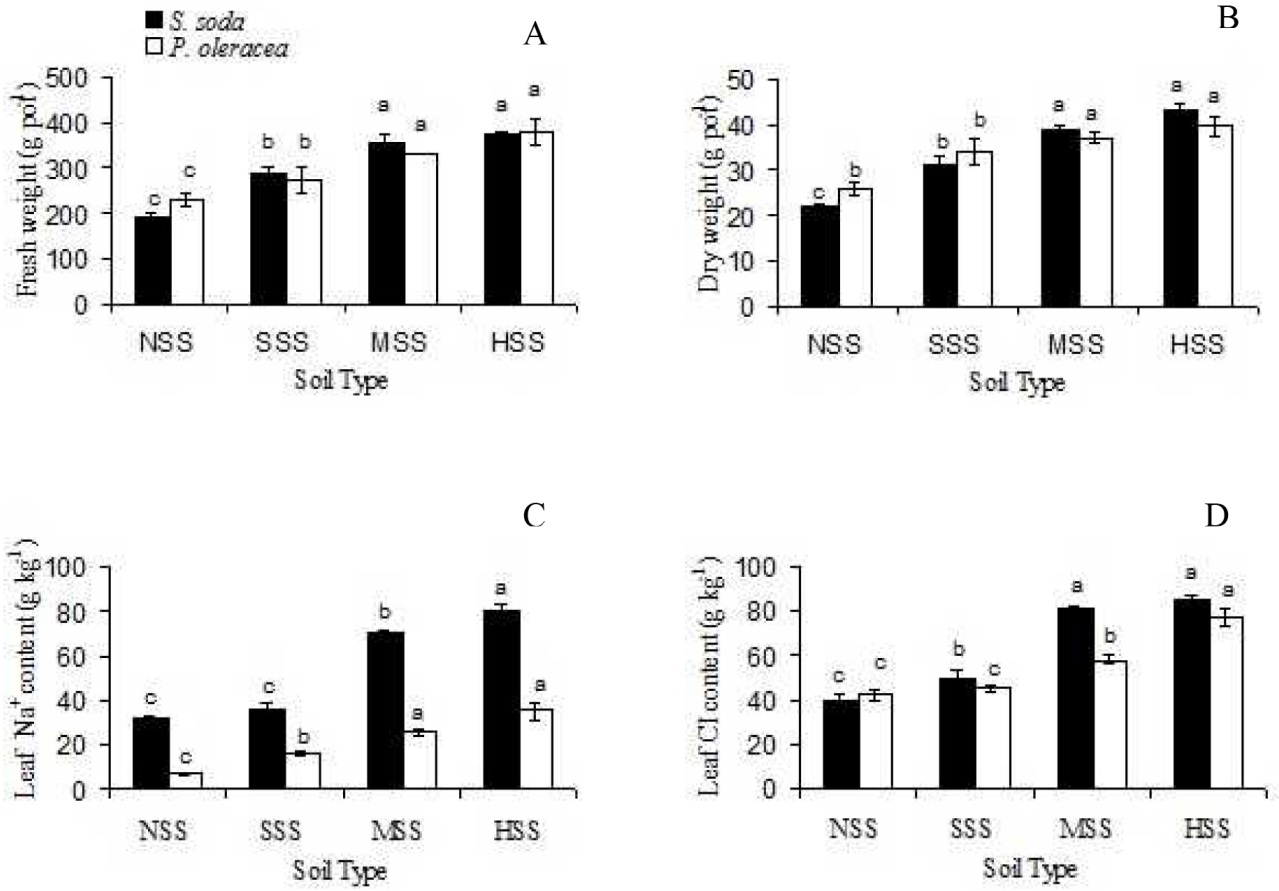

Figure 1. The fresh weight (A), dry weight (B), leaf Na^+ (C), and leaf Cl^- (D) of *S. soda* and *P. oleracea* plants for the four different salinity levels: nonsaline soil (NSS), slightly saline soil (SSS), moderately saline soil (MSS), and highly saline soil (HSS). Bars indicate the means of the six replicates ± standard error. Within species, bars marked with the same letter are not significantly different. Duncan's multiple range test, $P \leq 0.05$.

3.3. Uptake of Na^+ ions by halophytes (phytodesalination effect)

Both halophytes were determined to be quite effective in removing salt from saline soils. *S. soda* was capable of removing 30.6 mmol Na^+ pot^{-1} in NSS, 47.8 mmol Na^+ pot^{-1} in SSS, 119.4 mmol Na^+ pot^{-1} in MSS, and 151.4 mmol Na^+ pot^{-1} in HSS. *P. oleracea* was capable of removing 8.2 mmol Na^+ pot^{-1} in NSS, 23.8 mmol Na^+ pot^{-1} in SSS, 41.5 mmol Na^+ pot^{-1} in MSS, and 61.2 mmol Na^+ pot^{-1} in HSS. With regard to the mass removal of ions, we estimated that *S. soda* and *P. oleracea* were capable of removing 709 kg ha^{-1} and 286 kg ha^{-1}, respectively, from HSS (Figures 5A and 5B).

3.4. Discussion

The phytodesalination and production potentials (biomass production and ion uptake) of *S. soda* and *P. oleracea* were evaluated in four different soil types in 100-day pot experiments under controlled greenhouse conditions. The halophytes decreased EC and the Na^+ and Cl^- ion content of saline soils. The decrease is likely due to the uptake of ions by halophyte roots. Thus, the content of Na^+ and Cl^- in plants increased as salinity levels increased. Na^+ and Cl^- ions accumulated within the aerial portions of halophytes. The accumulation of salt ions increased with increases in soil salinity. An important finding from our research is that the halophytes displayed great tolerance to the deleterious influence of salinity by preserving the integrity of their cell membranes and their chlorophyll content. During the growth period, no indication of stress was observed through measurements of MDA, MSI, or the chlorophyll content of halophytes grown under saline conditions.

Our results agree with those reported by Ravindran et al. (2007), who evaluated the capacity of six halophytic species (*Suaeda maritima* Dum., *Sesuvium portulacastrum* L., *Clerodendron inerme* Gaertn., *Ipomoea pes-caprae* Sweet, *Heliotropium curassavicum* L., and *Excoecaria agallocha* L.) for desalinizing the upper 40 cm of soil in fields in India. The authors demonstrated that 120-day

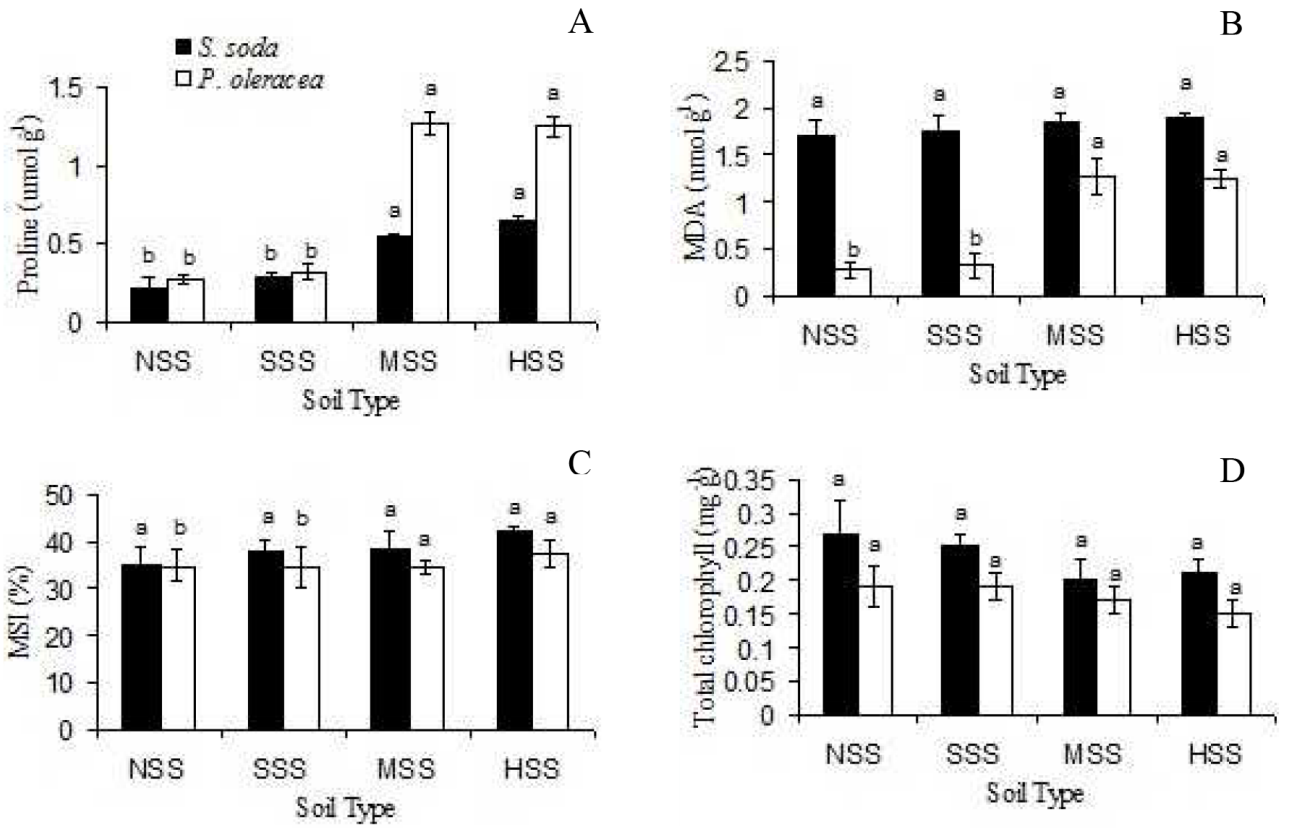

Figure 2. Contents of proline (A), MDA (B), MSI (C), and total chlorophyll (D) of *S. soda* and *P. oleracea* plants for the four different salinity levels: nonsaline soil (NSS), slightly saline soil (SSS), moderately saline soil (MSS), and highly saline soil (HSS). Bars indicate the means of the six replicates ± standard error. Within species, bars marked with the same letter are not significantly different. Duncan's multiple range test, $P \leq 0.05$.

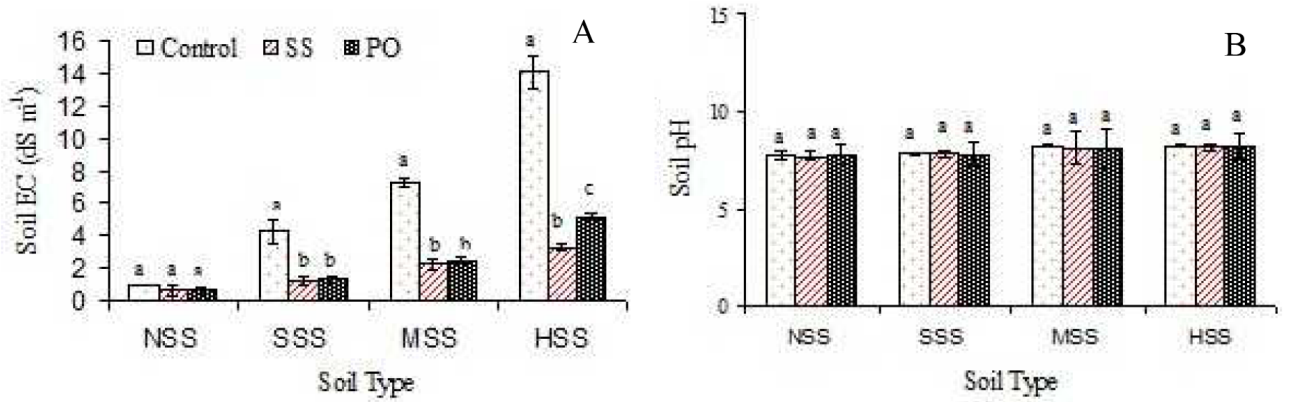

Figure 3. The EC (A) and soil pH (B) values of soils prior to planting (control) and following the harvest of *S. soda* (SS) and *P. oleracea* (PO) halophytes in nonsaline soil (NSS), slightly saline soil (SSS), moderately saline soil (MSS), and highly saline soil (HSS). Bars indicate the means of six replicates ± standard error. Within species, bars marked with the same letter are not significantly different. Duncan's multiple range test, $P \leq 0.05$.

cultivation using *S. maritima* and *S. portulacastrum* decreased the electrical conductivity of soils from 4.9 to 1.4 and 2.5 dS m^{-1}, respectively.

In the future, halophytes that are capable of accumulating sodium salts in their shoots could be successfully used for the removal of sodium from the

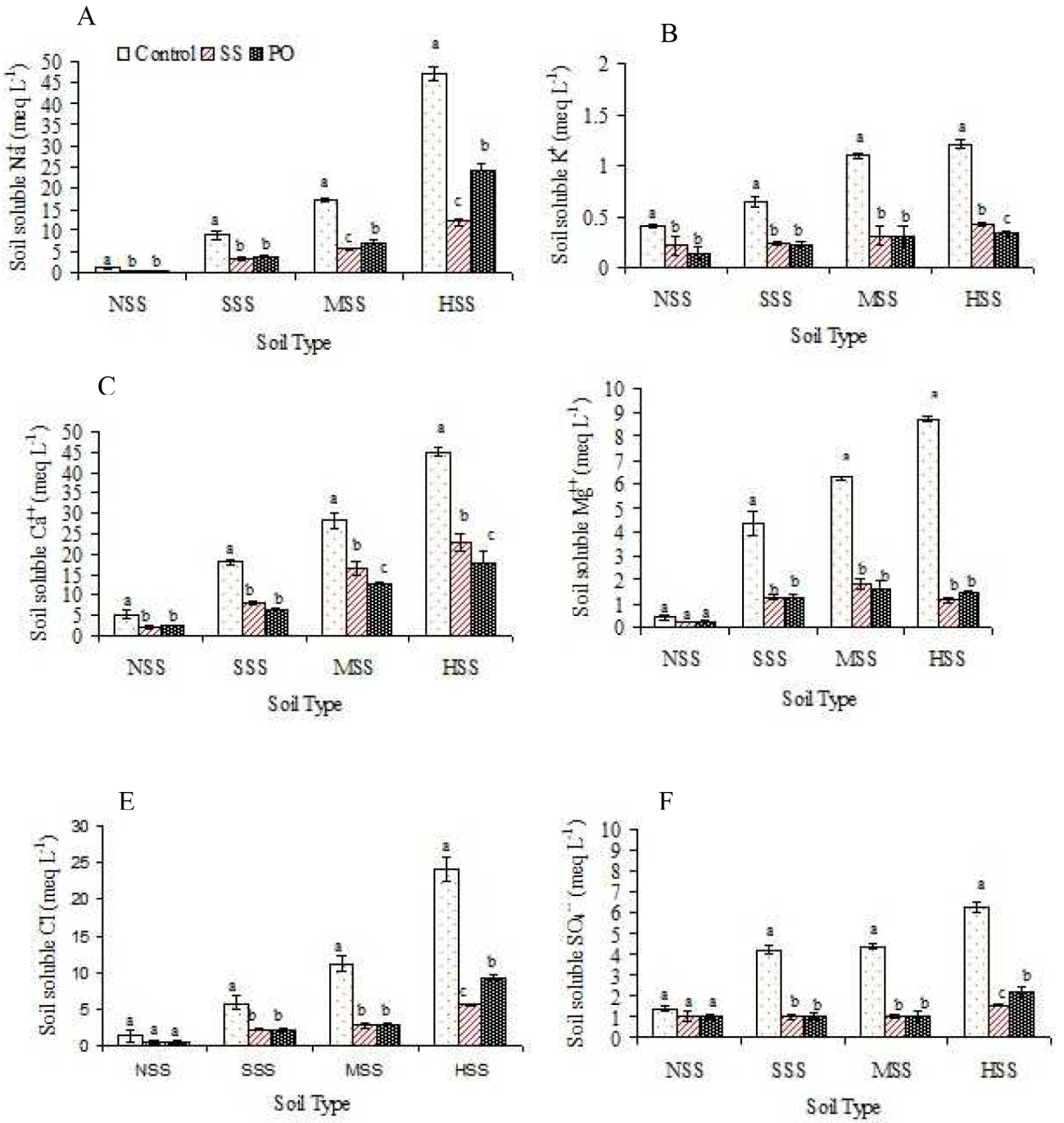

Figure 4. Soil soluble ion Na^+ (A), K^+ (B), Ca^{2+} (C), Mg^{2+} (D), Cl^- (E), and SO_4^{2-} (F) values of soils prior to planting (control) and following the harvest of *S. soda* (SS) and *P. oleracea* (PO) halophytes in nonsaline soil (NSS), slightly saline soil (SSS), moderately saline soil (MSS), and highly saline soil (HSS). Bars indicate the means of six replicates ± standard error. Within species, bars marked with the same letter are not significantly different. Duncan's multiple range test, $P \leq 0.05$.

substrate (soil) if plant shoots are harvested and removed from the field. Such a scenario would fit the poorly drained soils we used from the Harran Plain. Similar findings were also reported by Zhao et al. (2005) and Raphi et al. (2009).

In this work, we demonstrated that the accumulation of Na^+ and Cl^- ions increased in the leaves of both species under increasing salinity levels. Therefore, since they use salt ions for the osmotic adjustment of leaves and roots

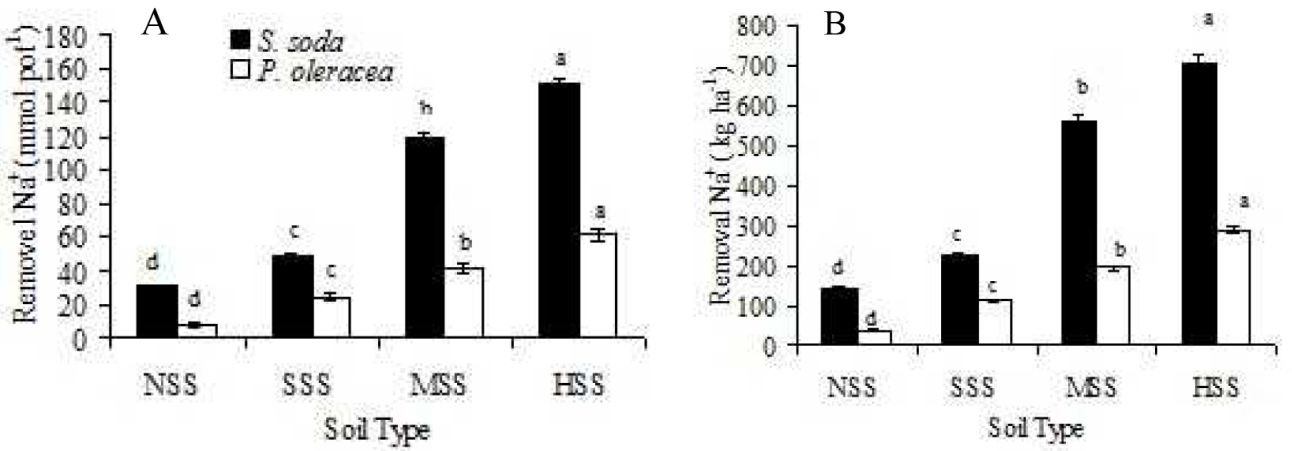

Figure 5. The removal of Na^+ from pots (A) and the adjusted removal capacity under field conditions (B) for *S. soda* and *P. oleracea* for the four soil types: nonsaline soil (NSS), slightly saline soil (SSS), moderately saline soil (MSS), and highly saline soil (HSS). Bars indicate the means of the six replicates ± standard error. Within species, bars marked with the same letter are not significantly different. Duncan's multiple range test, $P \leq 0.05$.

(Nguyen et al., 2004; Flowers and Colmer, 2015), salt accumulator plants could be very useful in saline areas.

Assuming this capacity can be matched by high biomass production, halophytic species could possibly be a biological solution for rehabilitating saline-sodic or salt-affected land. Halophytes potentially have the capability to extract significant quantities of salt from soils (Karakaş, 2013; Shabala, 2013). Such a finding, as well as additional attributes, may have led past researchers to suggest the co-cultivation of halophytes with crop plants and the growth of halophytes in salt-affected soils (Zorrig et al., 2012; Karakas et al., 2015).

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