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Halophytes relations to soil ionic composition

DUBRAVKA MILIĆ[1*], JADRANKA LUKOVIĆ[1], LANA ZORIĆ[1], JOVICA VASIN[2], JORDANA NINKOV[2#], TIJANA ZEREMSKI[2] and STANKO MILIĆ[2]

[1]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia and* [2]*Institute of Field and Vegetable Crops, Novi Sad, Serbia*

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Abstract: The concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} in the root and above-ground organs of three halophyte species (*Salicornia europaea*, *Suaeda maritima* and *Salsola soda*) as well as in the soil where they grew from maritime and inland saline areas were investigated. The aim of the research was to evaluate the capability of some halophyte species to absorb different cations and to find if a differentiation of salt accumulation between the populations from inland and maritime saline areas exists. In five analyzed localities (Tivatska solila, Ulcinj salina, Slano Kopovo, Melenci and Okanj), the external Na^+ concentrations exceeded those of the other investigated cations. The investigated halophytes accumulated more Na^+ than Mg^{2+}, Ca^{2+} and K^+ and more cations were recorded in the above-ground organs than in the root. The populations from maritime saline areas generally had higher cation concentrations than plants from inland saline areas.

Keywords: salt accumulation; *Salicornia europaea*; *Suaeda maritima*; *Salsola soda*.

INTRODUCTION

Soil salinization is considered one of the most important factors in land degradation.[1] Today, 20 % of the world's cultivated land and nearly half of all irrigated lands are affected by salinity.[2] NaCl is the major component of most saline soils.[3]

Generally, if a soil has a shallow water table then the salinity of the soil solution will be strongly affected by the salinity of the groundwater. Thus, in the main, the suitability of sites for plants may be based partly on whether the groundwater is too saline or toxic (*e.g.*, with a too low pH).[4]

* Corresponding author. E-mail: dubravka.milic@dbe.uns.ac.rs
[#] Serbian Chemical Society member.
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Land reclamation and rehabilitation could be achieved using salt tolerant species.[5] Halophytes generally use similar salt tolerance effectors and regulatory pathways that have been found in glycophytes, but that subtle differences in regulation account for large variations in tolerance or sensitivity.[6] Perennial deep-rooted plants in saline landscapes tend to access water mostly from the regions of the root-zone where the salinity of the soil solution is the lowest. Deep-rooted perennials are able to access water deeper in the soil profile during the dryer summer months, thus avoiding the highly saline soil solution near the surface, whereas more shallow-rooted annuals complete their lifecycle during periods when the salinity of the soil solution near the surface is lower than in the subsoil.[4]

Various halophytes are sensitive to various amounts of different ions. Generally speaking, Na^+ is more effective than K^+ and Cl^- more than SO_4^{2-} in promoting succulence. Many dicotyledonous halophytes are reported to grow optimally in NaCl concentrations ranging from 50 to 250 mM NaCl, while monocotyledonous halophytes grow optimally in the absence of salinity or sometimes in low (<50 mM) concentration of NaCl.[7] The most salt resistant plants are the salt accumulating halophytes, such as *Salicornia*, *Salsola* and *Suaeda*, that have a succulent structure.[8] In succulent halophytes, the vacuolar concentrations of Na^+ and Cl^- generally exceed the external concentrations. This appears to reflect a constitutive ability of halophytes to accumulate high ion concentrations.[9] Another group consists of the salt-localizing halophytes, including *Atriplex* and *Halimione*, in which the salt is localized in special hairs covering the stem and the leaves.[8] Halophytes are of great economic value and can fulfill almost every requirement of human beings, especially those related to food, fodder, fuel and medicines.[10] Moreover, halophytes are potentially ideal plants for phyto-extraction or phytostabilization applications of heavy metal polluted soils and of heavy metal polluted soils affected by salinity.[11,12]

The objective of this study was to determine the capability of halophyte species *Salicornia europaea* L., *Suaeda maritima* (L.) Dumort. *and Salsola soda* L. to absorb different cations and to determine if differentiation according to cation accumulation exists between the populations from maritime and inland saline areas.

EXPERIMENTAL

Soil and plant analyses were performed on samples from five localities: two from Montenegro (Tivatska solila and Ulcinj salina) and three from inland saline areas in the Pannonian plane in Serbia (Slano Kopovo, Melenci and Okanj) (Fig. 1). Ulcinj salina is the greatest saltern in Montenegro and Tivatska solila was previously a saltern, while the three localities in Pannonian plane are situated within the greatest agricultural area of Serbia.

Salicornia europaea, *Suaeda maritima* and *Salsola soda* were harvested in October 2006 and 2007. Simultaneously, soil samples were collected from the five analyzed localities. The plants were determined at the Department of Biology and Ecology, University of Novi Sad.

The content of water soluble salts, the soil pH value in suspension with water and conductivity (*EC*) in the soil were determined in accordance with ISO methods for soil quality.[13] The particle size distribution was determined in the < 2 mm fraction by the internationally recognized pipette method with Na-pyrophosphate preparation of the samples and a combination of sieving and the sedimentation fractionation model.[14]

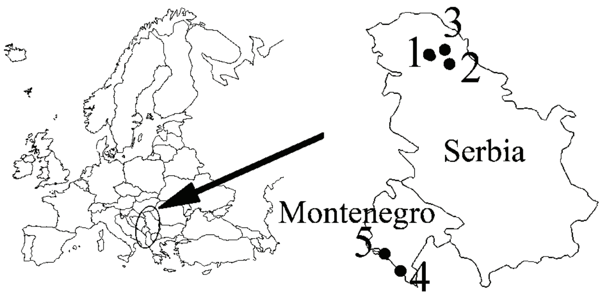

Fig. 1. Localities of the analyzed populations: 1 – Slano Kopovo, 2 – Melenci, 3 – Okanj, 4 – Ulcinj, 5 – Tivat.

The concentration of water soluble salts in soil was determined in soil water extract obtained by filtering soil suspension (soil paste) prepared by mixing 300 g of soil with 130––300 mL of deionized water in dependence on the water capacity of the soil sample. Analysis was subsequently performed by inductively coupled plasma–optical emission spectrometry, ICP-OES, (Varian Vista Pro-axial).

The plant roots were separated from the soil. The roots and the above-ground organs were washed three times with deionized water, oven-dried at 60 °C to constant mass and weighed. The concentrations of the cations in the plant tissues were determined by ICP-OES (Varian, Vista-Pro) after digestion in a mixture of 10 mL of HNO_3 (65 %) and 2 mL of H_2O_2 (30 %) using the microwave technique.

To avoid interferences and signal reductions due to ionization of Na and K, a Cs ionization buffer was used. A solution containing 2 % CsCl was added online to all solutions introduced into the ICP by pumping the solution into a "T" piece just before the nebulizer.

Data were statistically processed by analysis of the variance and means, standard errors and coefficients of variation, calculated using Statistica for Windows, version 10.0.[15] The significance of the differences in the measured parameters between the soil samples were determined using the Duncan test ($p \leq 0.05$), while the significance of differences in the cation concentrations in the plant organs from the inland and maritime saline areas were determined according to the *t*-test ($p \leq 0.05$). The general structure of the variability of the sample was established by principal component analysis (PCA), based on a correlation matrix. The overall differences between the compared groups are presented by Euclidian distances.

RESULTS

The texture characteristics of soil are presented in Table I. In all localities, except Tivat, clay was dominant and ranged from 28.6 to 52.6 %. The proportion of coarse sand was significantly higher in the Ulcinj locality, fine sand in the inland saline area Melenci and silt in Tivat.

TABLE I. Soil texture, mass %; different superscript letters, a–e indicate that the differences between the localities are significant according to the Duncan test ($p \leq 0.05$); the letter "a" represents the highest value, while the letter "e" the lowest; coefficients of variation are given in brackets

Soil component	Locality				
	Ulcinj saline	Tivatska solila	Okanj	Slano Kopovo	Melenci
Coarse sand	23.9±0.06^{a} (0.42)	3.7±0.01^{b} (0.03)	2.8±0.1^{c} (0.04)	3.7±0.1^{b} (0.03)	1.2±0.1^{d} (0.08)
Fine sand	24.3±0.01^{d} (0.04)	26.8 ±0.01^{b} (0.04)	16.6±0.01^{e} (0.06)	25.4±0.01^{c} (0.04)	37.0 ±0.01^{a} (0.27)
Silt	23.2±0.06^{e} (0.004)	40.4±0.01^{a} (0.02)	28.0±0.01^{b} (0.04)	24.4±0.01^{d} (0.04)	27.0±0.01^{c} (0.04)
Clay	28.6±0.99^{e} (0.06)	29.1±0.01^{d} (0.03)	52.6±0.01^{a} (0.02)	46.7±0.01^{b} (0.02)	34.8±0.01^{c} (0.03)

The soil at the inland saline areas contained small amounts of salts (0.6 mass %), whereas significantly higher amounts were detected in the soils at the maritime areas (0.8–1.5 mass %) (Table II). High *EC* values reflect the existence of an overlying salt crust. The soil at the Ulcinj locality had the highest conductivity (96 mS cm^{-1}). The soils at the localities from the inland saline area had the same *EC* values (6.6 mS cm^{-1}) and between these sites, there were no significant differences. The pH values ranged from 8.1 (Tivat) to 10.6 (Melenci). The Duncan test showed significant differences in the abundance of ions between all the analyzed soils from the five localities. The ion abundances of all the investigated cations were significantly greater in Ulcinj. The quantitative abundance of ions followed the order: sodium > magnesium > calcium > potassium.

Soil separation between analyzed samples was clearly observed based on the overall Euclidian distances, where localities from the inland saline area formed one cluster and were separated from the maritime ones (Fig. 2).

The sodium ion content for all species was higher than the Mg^{2+}, Ca^{2+} and K^{+} content (Fig. 3). A comparison between the three species showed that *Salicornia europaea* and *Suaeda maritima* were richer in Na^{+} and Mg^{2+} than *Salsola soda*. However, *Salsola soda* accumulated more K^{+} and Ca^{2+} than the other two species. The cation content in the above-ground organs of *Salicornia europaea*, *Suaeda maritima* and *Salsola soda* was greater than in the root. The populations from the maritime saline area generally had significantly higher cation concentrations than the plants from the inland saline area.

Principal components analysis (PCA) indicated the presence of three groups of analyzed parameters, which explained 86.2 mass % of the total variation (Table

TABLE II. Soil salinity – Salt composition; different superscript letters, a–e, indicate that the differences between the localities are significant according to the Duncan test ($p \leq 0.05$); the letter "a" represents the highest value, while the letter "e" the lowest

Parameter		Locality				
		Ulcinj saline	Tivatska solila	Okanj	Slano Kopovo	Melenci
Salts concentration, %		0.8±0.06^{b} (0.12)	1.5±0.06^{a} (0.07)	0.6±0.06^{c} (0.02)	0.6±0.06^{c} (0.02)	0.6±0.06^{c} (0.2)
Soil reaction, pH		8.2±0.01^{c} (0.12)	8.1±0.01^{d} (0.12)	9.4±0.01^{b} (0.11)	9.3±0.01^{b} (1.08)	10.6±0.01^{a} (0.09)
Conductivity, mS cm^{-1}		96.0±0.01^{a} (0.10)	46.3±0.01^{b} (0.22)	6.6 ±0.01^{c} (0.15)	6.6 ±0.01^{c} (0.15)	6.6±0.01^{c} (0.15)
Cation concentation, meq L^{-1}	Na^+	1031.3±0.1^{a} (0.001)	589.3±0.01^{b} (0.002)	55.5±0.01^{e} (0.02)	123.9±0.01^{d} (0.01)	239.4±0.01^{c} (0.004)
	K^+	28.8±0.01^{a} (0.03)	6.5±0.01^{b} (0.15)	0.1±0.01^{e} (8.33)	0.2±0.01^{d} (5.56)	0.6±0.01^{c} (1.53)
	Ca^{2+}	67.1±0.01^{a} (0.01)	42.9±0.01^{b} (0.02)	1.2±0.01^{d} (0.85)	1.9±0.01^{c} (0.53)	1.0±0.01^{e} (0.97)
	Mg^{2+}	201.9±0.01^{a} (0.005)	89.4±0.01^{b} (0.01)	1.2±0.01^{d} (0.84)	3.9±0.01^{c} (0.26)	0.8±0.01^{e} (1.26)

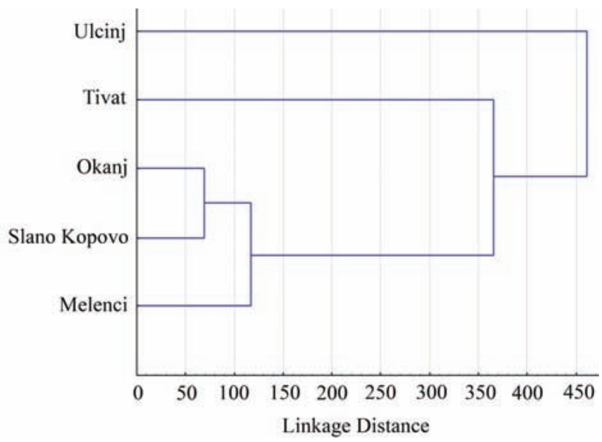

Fig. 2. Euclidian distances between the analyzed soil samples.

III). The most variable were the root and stem concentrations of Ca^{2+}, the Na^+ concentrations in the stem and Mg^{2+}concentrations in all plant organs. The first principal component explained 46.4 mass % of the variation. The second principal component explained 31.1 mass % of variation due to the variability of the Na^+ concentrations in the root and K^+ and Ca^{2+} concentrations in the leaves. The third principal component explained 8.7 mass % of variation. Parameters that did not contribute significantly to the total variation were the accumulations of K^+ in the root and stem and the Na^+ concentrations in the leaves. According to the type of variability, the examined populations were grouped by PCA (Fig. 4). The projection of the cases for the first two components showed that populations from inland and maritime saline areas could be clearly separated according to the type of variation in the accumulation of cations.

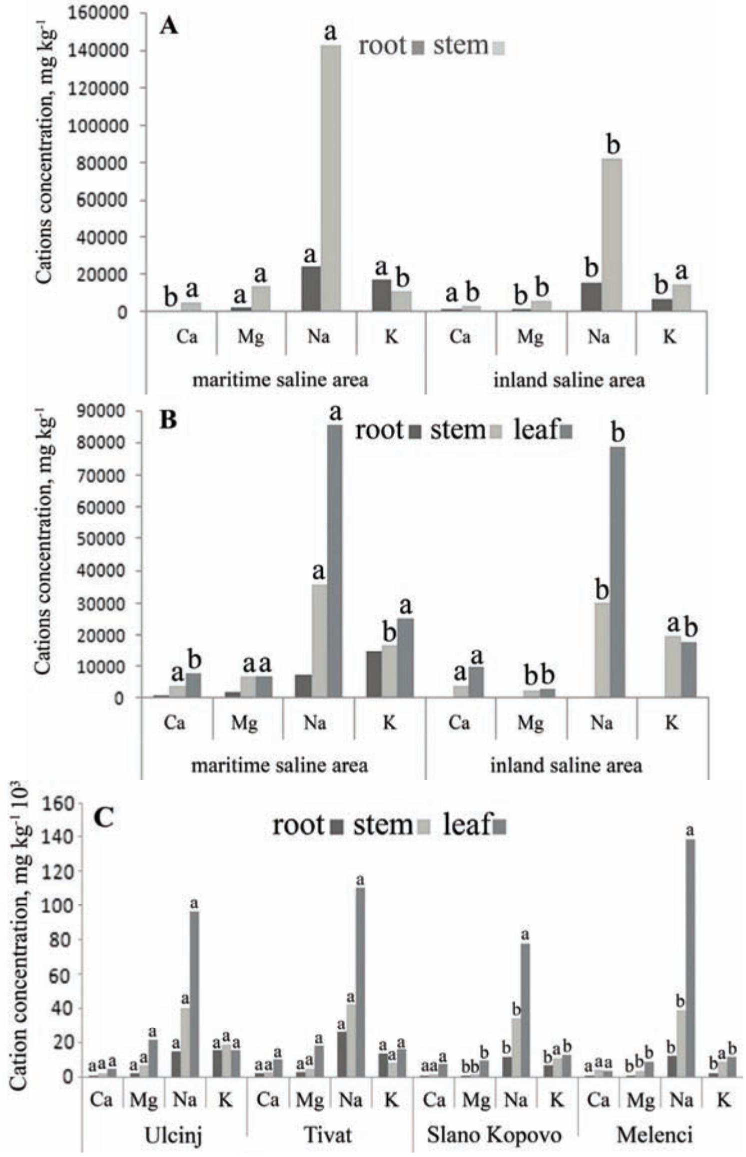

Fig. 3. Mean Na^+, K^+, Ca^{2+} and Mg^{2+} contents in root, stem and leaf of A – *Salicornia europaea*, B – *Salsola soda* and C – *Suaeda maritima* from the inland and maritime saline areas. Letters from "a" to "e" indicate differences in cation concentrations between the plant organs from the inland and maritime saline areas. The same letter means that the differences are not significant according to the *t*-test ($p \leq 0.05$); the letter "a" represents the highest value, while the letter "e" the lowest; MSA – maritime saline area, ISA – inland saline area.

DISCUSSION

Under natural conditions, halophytes accurately reflect the chemical composition of the soil as well as the intensity and type of salinity.[16,17]

TABLE III. Factor analyses of the cation concentrations in the halophytic species from the inland and maritime saline areas; * marked numbers are statistically significant at $p \leq 0.05$

Part	Ion	Factor 1	Factor 2	Factor 3
Root	Na^+	–0.622314	0.733941*	–0.226497
	K^+	–0.685478	0.480832	0.466223
	Ca^{2+}	–0.717438*	0.347608	–0.430767
	Mg^{2+}	–0.825518*	0.483519	0.085686
Stem	Na^+	–0.732384*	0.313893	–0.168400
	K^+	–0.590531	–0.526443	0.540335
	Ca^{2+}	–0.760969*	–0.472099	–0.232195
	Mg^{2+}	–0.898387*	0.173187	0.216213
Leaf	Na^+	–0.523447	–0.681560	–0.282737
	K^+	–0.378400	–0.894610*	0.163398
	Ca^{2+}	–0.327730	–0.814599*	–0.242078
	Mg^{2+}	–0.848353*	–0.221580	0.014006
Cumulative percentages of the vectors		46.41	77.50	86.23

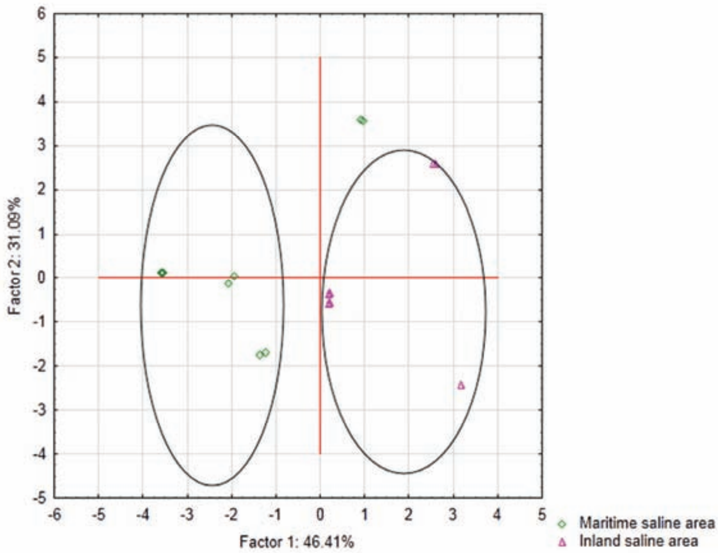

Fig. 4. The projection of the cases of the first two components of the principal component analysis.

In most saline environments,[8] including the five localities analyzed in the present study, the external Na^+ concentrations exceed those of the other investigated cations. Soils are considered to be salinized when a soil saturation extract has an *EC* value of 4 dS m^{-1} or greater.[18] In the investigated localities, the *EC* values ranged from 6.6–96 dS m^{-1}, which confirmed that the localities were strongly salinized.

Many species of Chenopodiaceae accumulate large amounts of Na^+ and Cl^- when the external salinity is high.[19–21] The investigated halophytes accumulated more Na^+ than Mg^{2+}, Ca^{2+} and K^+. Keiffer and Ungar [22] obtained similar result for *Atriplex prostrara*, *Hordeum jubatum*, *Salicornia europaea* and *Spergulatria marina*. The calcium contents in the plant organs of *Salsola soda* were higher than the magnesium contents, while in *Salicornia europaea* and *Suaeda maritima* the opposite was found. The calcium content decreased as the soil salinity increased, probably because of a competitive effect with sodium.[23] The halophytes investigated in the present research accumulated cations more in aboveground organs than in the root. This feature could be associated with the efficiency of such plants to deliver ions into the vacuoles.[24] Many halophytic species are characterized by their large vacuoles. Vacuoles occupy 77 % of the leaf mesophyll cells of *Suaeda maritima*,[25] and are capable of accumulating salts to concentrations higher than 500 mM.[26]

Principal component analysis showed that the populations from the inland and maritime saline areas had different types of variability in ion accumulation and distribution in their organs, and that they could be clearly separated. These differences were probably also induced by the differences in the soil composition of their sites.

CONCLUSIONS

Soil salinization is considered one of the most important factors in land degradation. The results of this study indicate that halophyte species *Salicornia europaea*, *Suaeda maritima* and *Salsola soda* may accumulate significant amounts of salt from salt-affected soil and therefore remediate land to the point where native plants could invade and become established, or the site could be returned to agricultural productivity. Other important benefits from bioremediation include erosion control, reduced salt in the soil and conservation of wildlife.

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ИЗВОД

УТИЦАЈ ЈОНСКОГ САСТАВА ЗЕМЉИШТА НА ХАЛОФИТЕ

ДУБРАВКА МИЛИЋ[1], ЈАДРАНКА ЛУКОВИЋ[1], ЛАНА ЗОРИЋ[1], ЈОВИЦА ВАСИН[2], ЈОРДАНА НИНКОВ[2], ТИЈАНА ЗЕРЕМСКИ[2] и СТАНКО МИЛИЋ[2]

[1]*Департман за биологију и екологију, Природно математички факултет, Универзитет у Новом Саду* и [2]*Институт за ратарство и повртарство, Нови Сад*

У овом раду су испитиване концентрације Na^+, K^+, Ca^{2+} и Mg^{2+} у корену и надземним биљним органима код три халофитске врсте (*Salicornia europaea*, *Suaeda maritima* и

Salsola soda), као и састав земљишта на којима ове биљке расту са маритимних и континенталних халобиома. Циљ рада је био да се утврди способност халофитских врста да апсорбују различите катјоне, као и да се испита да ли постоји диференцијација популација са маритимних и континенталних халобиома у односу на способност њихове акумулације соли. У земљишту код пет анализираних локалитета (Тиватска солила, Улцињска солана, Слано Копово, Меленци и Окањ) је констатована већа концентрација Na^+ у односу на друге катјоне. Истраживане халофитске врсте акумулирају више Na^+ него Mg^{2+}, Ca^{2+} и K^+. Такође, већа концентрација катјона забележена је у надземним органима, него у корену. У вегетативним органима популација са маритимних халобиома констатована је већа концентрација катјона у поређењу са биљкама са континенталних халобиома.

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REFERENCES

1. I. Rogel, S. Ortiz, A. Alcaraz, *Geoderma* **99** (2001) 81
2. J. D. Rhoades, J. Loveday, *Salinity in irrigated agriculture*, in *Irrigation of agricultural crops*, B. A. Steward, D. R. Nielsen, Eds., American Society of Agronomists, Madison, WI, 1990, p. 1089
3. P. E. Verslues, M. Agarwal, S. Katiyar-Agarwal, Z. Jianhua, Z. Jian-Kang, *Plant J*. **45** (2006) 523
4. S. J. Bennett, E. G. Barrett-Lennard, T. D. Colmer, *Agric. Ecosyst. Environ.* **129** (2009) 349
5. H. N. Le Houérou, Salt-tolerant plants for the arid regions of the Mediterranean isoclimatic zone, in Towards the rational use of high salinity tolerant plants, Vol. 1, H. Lieth, A. Al Masoom, Eds., Kluwer Academic Publishers, Dordrecht, 1993, p. 403
6. J. K. Zhu, *Plant Physiol*. **124 (**2000) 941
7. T. J. Flowers, T. D. Colmer, *New Phytol*. **179** (2008) 945
8. H. R. Krüger, N. Peinemann, *Vegetation* **122** (1996) 143
9. T. J. Flowers, *Plant Soil* **89** (1985) 41
10. A. Hameed, M. A. Khan, *Karachi Univ. J. Sci*. **39** (2011) 40
11. E. Manousaki, N. Kalogerakis, *Ind. Eng. Chem. Res.* **50** (2011) 656
12. D. Milić, J. Luković, J. Ninkov, T. Zeremski-Škorić, L. Zorić, J. Vasin, S. Milić, *Cent. Eur. J. Biol.* **7** (2012) 307
13. ISO 10390, *Soil quality – Determination of pH*, 1994
14. Handbook of JDPZ research methods, determination of physical soil properties, Novi Sad, 1997, p. 17 (in Serbian)
15. StatSoft, Inc., Statistica (data analysis software system), version 10.0, 2011, www.statsoft.com
16. V. A. Kovda, C. Van den Berg, R. M. Hagan, Eds., *Irrigation, drainage and salinity*, Hutchinson, FAO, Unesco, London, 1973, pp.1–150
17. C. V. Malcolm, in *Biology and utilization of shrubs*, C. M. McKell, Ed., Academic Press, San Diego, CA, 1989, pp. 553–574
18. M. Treshow, *Environment and Plant Response*, McGraw Hill, New York, 1970, p. 345
19. R. Albert, *Oecologia* **21 (**1975) 57
20. I. A. Ungar, *Actualites Botaniques* **125** (1978) 95
21. T. J. Flowers, M. A. Hajibagheri, N. J. W Clipson, *Q. Rev. Biol*. 61 (1986) 313
22. K. H. Keiffer, I. A. Ungar, *Wet Ecol Manag.* **9** (2001) 469

23. W. H. Van der Molen, in *Prognosis of Salinity and Alkalinity*, Report of an Expert Consultation Rome, 1975, FAO, Rome, Italy, 1976, pp. 31–52
24. Z. Dajić, in *Physiology and molecular biology of stress tolerance in plants*, K. V. Madhava Rao, A. S. Raghavendra, K. J. Reddy, Eds., Springer, Dordrecht, 2006, p. 41
25. M. A. Hajibagheri, J. L. Hall, T. J. Flowers, *J. Exp. Bot.* **35** (1984) 1547
26. M. N. H. Dracup, H. Greenway, *Plant Cell Environ.* **8** (1985) 149.