Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 2, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TRACING MINERAL PATHWAYS IN HALOPHYTES: FROM ROOTS TO AERIAL PARTS

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Abstract

Soil salinization is a growing global challenge, affecting agricultural productivity and ecosystem stability. This phenomenon results from both human activities, such as improper irrigation and deforestation, and climate change-driven factors like rising temperatures and altered precipitation patterns. With increasing soil degradation, effective and sustainable solutions are urgently needed.

Halophytic plant species offer a promising strategy for mitigating soil salinization. These plants can naturally tolerate high salt concentrations by absorbing and transporting excess salts to specific tissues, thereby reducing soil salinity. Additionally, halophytes hold economic potential, as they can be utilized in various industries, including bioenergy, pharmaceuticals, and agriculture. Some species provide alternative food sources or raw materials for cosmetics and medicine.

By integrating halophytes into land management practices, degraded soils can be rehabilitated while generating valuable resources. However, further research is needed to optimize species selection and large-scale implementation. This study highlights the extraction potential of halophytes in addressing soil salinization and emphasizes their role in sustainable land use strategies.

Key words*: elemental analysis, inductively coupled plasma with mass spectrometry, phytoremediation, salinization.*

INTRODUCTION

To meet the growing global demand for food, one of the most effective solutions may be to increase land productivity. However, agricultural intensification involves high costs due to high water, energy, and pesticide consumption, as well as the depletion of soil nutrients and the negative impact on ecosystem services.

Poor management of agricultural practices increases soil salinity and water resource depletion, leading overall to the aridification of large areas. Soil salinity is a well-known abiotic stress factor that can hinder plant growth and development, reducing agricultural yield (Amombo et al., 2018; Barros et al., 2021; Rahman et al., 2021). One alternative solution may be to remediate these lands by using potential new crops, such as halophytes (Hasanuzzaman et al., 2014; Ahmadi et al., 2022). Through their morphological, physiological and anatomical characteristics, they have evolved the ability to tolerate high concentrations of sodium chloride (NaCl) (Rahman et al., 2021). Halophytes tolerate sodium (Na^+) and potassium (K^+) ions due to their capacity to sequester cytotoxic Na^+ in the vacuoles of root and leaf cells – a trait conferred by the constitutive expression of tonoplast Na^+/H^+ exchangers NHX complemented by the control of slow and fast vacuolar ion channels to prevent Na+ leakage back into the cytosol (Panta et al., 2014).

In this context, this paper presents a possible model of use of three halophyte species - *Portulaca oleracea, Festuca arundinacea Limonium sinuatum*, highlighting the translocation of mineral elements from soils to different parts of them.

The World Health Organization has referred to purslane as a 'Global Panacea' and it is regarded as one of the most valuable medicinal herbs (Sultana & Rahman, 2013) due to its high content in vitamins, amino acids, minerals and polysaccharides (Petropoulos et al., 2016; Bekmirzaev et al., 2021; Jalali & Ghasemzadeh Rahbardar, 2022; Srivastava et al., 2023). Because of its remarkable ability to adapt and thrive under adverse environmental conditions and diverse climatic zones, it is an invasives species, with a cosmopolitan distribution. *Portulaca oleracea* evolved a unique type of photosynthesis as a response to drought and salt stress. Even though it is agreed that the species presents a C4 metabolism and the leaf structure displays a Kranz anatomy, with the mesophyll cells arranged to form a ring around the bundle-sheath cells, it can swith to CAM metabolism, even in the absence of photosynthetic stems, making it a CAM-facultative (D'Andrea et al., 2014; Sdouga et al., 2019; Wang et al., 2023). Purslane cultivated under high salinity stress accumulates proline, an osmoprotectants, and shows an enhanced capacity of the antioxidative system by increasing the activity of ROS - scavenging enzymes and lowering the lipid peroxidation (Yazici et al., 2007; Ocampo & Columbus, 2012). Studies have highlighted a high potential for the phytoremediation of contaminated soils, as the species is capable of hyperaccumulating and removing from the excess minerals (Bekmirzaev et al., 2021) and heavy metals (Negi, 2018; Elshamy et al., 2019; Londe et al., 2025).

Another species included in this study, *Festuca arundinacea* is a member of the Poaceae family, and, along with other members of the genus *Festuca* (*F. ovina* L., *F. rubra* L.), it is commonly used for lawns, due to its resistance to abiotic stress factors such as high temperatures, water deficiency and saline stress (Szekely-Varga et al., 2023). *Festuca arundinacea,* commonly known as 'tall fescue', even though is it not a true halophyte, it has a moderate resistance to salinity stress (Díaz et al., 2013; Kaplan et al., 2017; Batog et al., 2023). Moreover, additional to its use in lawns, it can be cultivated for biocomposite and bioethanol production (Batog et al., 2023).

Limonium (family Plumbaginaceae) is an almost globally distributed genus of salt-tolerant species, with its primary center of diversity located in the Mediterranean area (Malekmohammadi et al., 2017; Koutroumpa et al., 2018; Jēkabsone et al., 2023). Those species are commonly known as sea lavender. Research on different *Limonium* species have shown that salinity tolerance relies primarily on the active movement and buildup of ions within the leaves, accompanied by the production of proline and soluble sugars, which function as compatible solutes to maintain an osmotic balance (Al Hassan et al., 2016; González-Orenga et al., 2020). Moreover, the species of this genus possess salt glands that are also found in halophytic members of other families, such as Frankeniaceae and Tamaricaceae, making them capable of secreting various ions (Ca^{2+}, Na^{+}, K^{+}, HCO^{3-}, Cl^{-}) and trace elements (Ni, Pb, Mn, Fe, Cu, As, Cd, etc.) (Flowers et al., 2010; Caperta et al., 2020). The species used in this study, *Limonium sinuatum*, is commonly cultivated for its used as dried flowers (Xu et al., 2021). The dwarf varieties of *Limonium* are ideal for flower beds and borders, while the taller varieties are used in small groups and mixed borders, especially in areas with poor soils and reduced precipitation (Toma, 2009)

Therefore, the study aims to present the translocation of the elements from saline soils in different parts of those plant species during their lifecycle, for future applications in soil remediation and their subsequent recovery.

MATERIALS AND METHODS

Biological material

The biological material consisted of seeds of *Portulaca oleracea* subsp. *sativa, Festuca arundinacea* L., and *Limonium sinuatum* L. These were sown, to produce 4-week-old seedlings, which were then transplanted into the experimental plots in Tulcea (non-saline soil) and Dâmbovița (saline soil) areas of Romania.

Depending on the species, the following parameters were monitored in April, May and July in both experimental plots: biometric measurements (plant height, number of leaves, flowers, stems, tillers and inflorescences, elemental content (CHN and minerals) of both plants and soil; as well as soil electrical conductivity and pH.

The determination of the soil reaction (pH) was carried out using a potentiometric method, by directly measuring the concentration of hydronium ions in the soil suspension, with a soil-to-water ratio of 1:2.5 (in accordance with standard SR ISO 10390:2015). For this, 10 g of soil were weighed into a 50 mL bottle, to which 25 mL of double-distilled water were added, forming an aqueous solution. The suspension was subjected to agitation for 1 hour on a horizontal shaker. After agitation, the sample was left to rest for 1 hour.

Before measurement, it was agitated again for 5 minutes. The pH was read directly in the sample in the suspension.

The determination of the specific electrical conductivity (EC) was carried out in accordance with the reference standard: - SR ISO 11265 + A1: 1998 Soil quality.

The soil sample was dried in the atmosphere and sieved through a 2 mm strainer. Ten grams of soil were weighed and 50 ml of ultrapure water were added. The samples were shaken for 1 hour at 15 rpm. After decantation, the suspension was filtered, and the electrical conductivity was measured directly in the prepared sample.

Elemental analysis (CHN) - preparation of soil and plant samples

Soil and plant samples were collected from the experimental plots, dried, and ground to a size of 250 µm. For sample analysis, tin capsules (8 x 5 mm) were used into which 0.01-0.02 g of each sample was weighted. The same protocol was applied for the plant samples.

For the calibration curve of the equipment, Cystine was used as a standard. The total nitrogen (N) and carbon (C) content of the cystine/standard were 11.64 and 29.95%, respectively. Five standards were weighed, of different concentrations ranging from 0.5 to 2.5 mg.

Sample analysis

Sample analysis was performed using the EA3100 elemental analyzer.

The mineral composition of the samples (both plat and soil) was determined according to the method described by Constantin et al., 2023.

Regarding the biometric measurements, they were carried out on a number of five plants per experimental variant. Thus, depending on the species, the following parameters were analyzed: plant height (H), number of leaves (Nf), number of floral stems, number of inflorescences, number of tillers and height of floral stem

RESULTS AND DISCUSSIONS

Results on the growth and development of Limonium sinuatum

Regarding the growth and development of *Limonium sinuatum* in the two experimental lots, the results are included in Figure 1.

In terms of plant height, the growth on saline soil was comparable to the data recorded on the non-saline soil in Tulcea. Differences were relatively minor, up to 10 cm, in the last stage of data collection. Thus, in Tulcea, a height of approximately 50 cm was recorded, while 38 cm was recorded in the Dâmbovița area.

Concerning average leaves/plant, it can be seen that there were no major differences (10-11 leaves), during the first two months of the experiment. Nevertheless, this difference became significant in the last stage of data collection (38 leaves/plant in the Dâmbovița lot, compared to 86 leaves/plant in the Tulcea reference plot).

The formation of floral stems began in July in both experimental lots, with their number increasing on average to 10 and 12 stems/plant in the Dâmbovița and Tulcea plots, respectively. The height of the floral stem was recorded in both experimental plots, increasing on average to 26 and 28 cm.

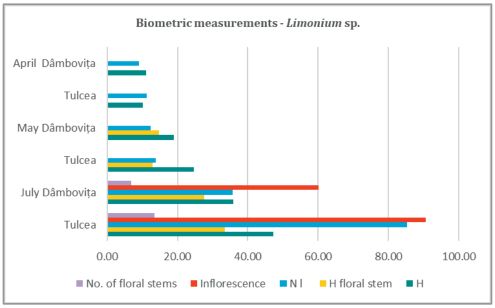

Figure 1. Dynamics of growth and development parameters of *Limonium* sp.

Similarly, the number of flowers grouped in inflorescences was counted in July, averaging between 60-83 flowers grouped in inflorescences/plant, the differences being significantly different between the two plots.

Results on the growth and development of Festuca arundinacea

Regarding the growth and development of *Festuca* sp. in the two experimental plots, the results are presented in Figure 2. Therefore, regarding:

Plant height (H) - it can be observed that the species developed similarly in both experimental plots, with the plant height reaching a maximum of 27.4 cm;

Number of leaves (Nl) - the average is similar in the two experimental plots in the first two months, reaching a maximum number of 4 leaves in the case of plants from the Dâmbovița experimental plot;

The number of tillers (siblings) - similar to the plant height, this trait/ parameter did not differ significantly from the values recorded in the experimental plot in Tulcea.

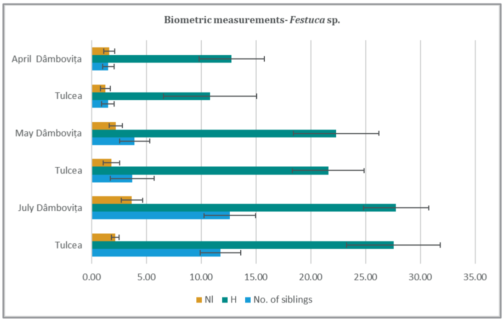

Figure 2. Dynamics of growth and development parameters of *Festuca* sp.

***Results on the growth and development of Portulaca oleracea* sp.**

Regarding the growth and development of the *Portulaca* sp. species in the two experimental batches, the results are shown in Figure 3. Therefore, regarding:

Plant height (H) - Compared to the data recorded on the non-saline soil in Tulcea, the height was generally similar. However, in May, the maximum height recorded in the Tulcea plot was 48 cm, while in the Dâmbovița plot it reached only 20 cm;

Number of leaves (Nl) - in the first two months, the number of leaves was similar (15-16 leaves). However, notable differences appeared in the final month (18 leaves Dâmbovița lot compared to 108 in the Tulcea reference plot);

Number of inflorescences - the formation of buds began in May in the Dâmbovița area, while in Tulcea in occurred during the months of June and July, likely due to geographical differences. However, on the saline soil the number of inflorescences continued to increase to an average number of approximately 37 per plant.

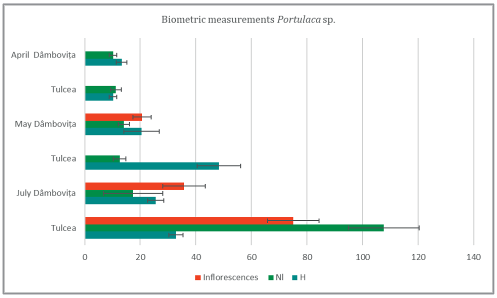

Figure 3. Dynamics of growth and development parameters of *Portulaca* sp.

All three species have showed, at least in terms of height, a stunned growth in the saline-soil plot in Dâmbovița, compared to the Tulcea plot.

Results of the analysis of ~~the~~ mineral composition in plants and soil

The results of mineral analysis of the plants grown in the experimental plot in Dâmbovița are presented in Table 1, while those from the experimental lot in Tulcea are shown in Table 2.

Table 1. Results regarding the mineral content of cultivated plants – experimental plot Dâmbovița

Sample	*Na g/Kg*	*SD*	*Mg [g/Kg]*	*SD*	*K [mg/Kg]*	*SD*	*Ca [mg/Kg]*	*SD*
Limonium leafs	*34.93*	*1.63*	*4.812*	*0.210*	*12.35*	*0.64*	*6.46*	*0.57*
Limonium roots	*14.37*	*0.86*	*2.774*	*0.094*	*17.32*	*1.18*	*3.23*	*0.10*
Festuca leafs	*26.62*	*1.38*	*2.672*	*0.067*	*4.51*	*0.18*	*6.90*	*0.63*
Festuca roots	*10.05*	*0.43*	*1.635*	*0.038*	*5.60*	*0.42*	*6.77*	*0.60*
Portulaca leafs	*53.75*	*2.47*	*9.908*	*0.443*	*17.46*	*0.99*	*21.64*	*1.06*
Portulaca stems	*46.35*	*1.95*	*4.098*	*0.175*	*41.99*	*1.69*	*10.61*	*0.56*
Portulaca roots	*44.62*	*1.99*	*6.489*	*0.277*	*43.81*	*1.99*	*7.95*	*0.55*

By comparing the results from the two experimental lots, the following observations can be made:

- High concentrations of sodium were found in the leaves of *Limonium* sp. and in all parts of *Portulaca* sp., especially in the leaves;
- The highest concentrations of magnesium were detected in the leaves of *Portulaca* sp.;
- *Portulaca* sp. also contains the highest concentration of potassium - 40 mg/kg in roots and stems; Simillarily, calcium is also found in large quantities in the leaves.

Within the Tulcea experimental group, the results indicate that the stems of *Limonium* sp. and the roots of *Portulaca* sp. are rich in sodium, even when grown in non-saline conditions. Similar levels of magnesium were also determined in the leaves and stems of the same species. The highest concentrations of potassium (77 g/kg) and calcium (20 g/kg) were found in stems of *Portulaca* sp.

Table 2. Results regarding the mineral content of cultivated plants – Tulcea experimental plot

Planta	Na [g/Kg]	SD	Mg [mg/Kg]	SD	K [g/Kg]	SD	Ca g/kg	SD
Limonium leafs	*12.36*	*0.79*	*10.21*	*0.64*	*34.65*	*2.57*	*9.64*	*0.74*
Limonium roots	*2.21*	*0.10*	*2.08*	*0.10*	*21.51*	*1.50*	*2.08*	*0.13*
Festuca leafs	*1.29*	*0.06*	*2.91*	*0.12*	*23.80*	*1.41*	*4.24*	*0.15*
Festuca roots	*1.10*	*0.04*	*1.65*	*0.06*	*7.46*	*0.59*	*10.22*	*0.47*
Portulaca stems	*4.67*	*0.31*	*11.64*	*0.72*	*77.38*	*4.39*	*20.00*	*0.91*
Portulaca roots	*12.81*	*0.64*	*6.36*	*0.32*	*19.53*	*1.10*	*8.98*	*0.62*

The results of soil analysis from the Dâmbovița and Tulcea experimental plots, before and after planting halophyte species, are presented in Table 3.

Table 3. Results regarding the analysis of minerals from cultivated soils

Soil sample	Na g/kg	SD	Mg g/kg	SD	K g/kg	SD	Ca g/kg	SD
Soil *Festuca* sp. Tulcea	0.13	0.00	6.240	0.080	3.29	0.30	29.16	0.79
Soil *Portulaca* sp. Tulcea	0.14	0.00	5.600	0.024	3.55	0.20	17.61	0.44
Soil *Limonium* sp. Tulcea	0.11	0.00	5.255	0.066	2.77	0.13	18.44	0.63
Initial soil Tulcea	0.08	0.00	5.243	0.028	2.93	0.12	18.07	0.57
Soil *Festuca* sp. Dâmbovița	1.54	0.05	12.424	0.113	2.00	0.11	31.68	0.62
Soil *Portulaca* sp. Dâmbovița	1.87	0.06	12.515	0.142	1.98	0.11	31.34	0.35
Soil *Limonium* sp. Dâmbovița	0.98	0.03	12.240	0.098	2.23	0.09	30.26	0.59
Initial soil Dâmbovița	2.05	0.05	12.544	0.081	2.27	0.08	29.76	0.45

Within the experimental plot in Dâmbovița, the results show a significant decrease in sodium concentration when cultivating *Limonium* sp., from 2 g/kg to 0.98 g/kg. In the case of calcium, a notable increase in concentration is observed only when cultivating the *Festuca* sp. Regarding magnesium and potassium, cultivation of the species in different conditions did not result in significant changes in their concentrations.

Within the experimental plot in Tulcea, according to the results obtained, significant changes were recorded in the calcium concentration when using *Festuca* sp.

Results on C, H, N analysis of soils and plants

The results of the C, H, N analysis for the soils and plant samples in the experimental plots are presented below in the form of tables and graphs.

The soil analysis results are presented in Table 4. According to the results obtained, a decrease in the amount of C, H, N compared to the initial (pre-planting) soil can be observed, demonstrating the hypothesis that the plants extracted these elements from the soil as they grew and developed.

Table 4. The C, H, N content of the soil in the Dâmbovița area

Sample	N %	C %	H %
Initial soil Tulcea	0.09 ± 0.06	1.21 ± 0.73	0.25 ± 0.15
Soil after cultivation Limonium sp.	0.12 ± 0.01	1.55 ± 0.07	0.31 ± 0.02
Soil after cultivation Portulaca sp.	0.15 ± 0.06	2.16 ± 1.04	0.42 ± 0.22
Soil after cultivation Festuca sp.	0.11 ± 0.06	1.62 ± 0.76	0.29 ± 0.14
Initial soil Dâmbovița	0.064 ± 0.01	1.608 ± 0.13	0.269 ± 0.02
Soil after cultivation Limonium sp.	0.054 ± 0.01	1.540 ± 0.03	0.227 ± 0.04
Soil after cultivation Festuca sp.	0.031 ± 0.02	1.495 ± 0.04	0.199 ±0.02
Soil after cultivation Portulaca sp.	0.051 ± 0.00	1.528 ± 0.07	0.197 ±0.03

For each species of plant, a detailed analysis of the C, H, N content found in different organs was made, as follows:

Festuca (root and leaves);

Portulaca (root, stems and leaves);

Limonium (root and leaves).

For *Portulaca* sp., the highest N content was determined in the shoots from the experimental lot in Dâmbovița. The highest value for C - 39.8% - was found in the roots from Tulcea, while the highest hydrogen (H) content, of 5.3%, was also determined at root level in the plants cultivated in Tulcea. In case of *Festuca arundinacea,* a carbon (C) content of 3.6% C was determined in the leaves of the plants cultivated in Dâmbovița. The C% content of in the roots was 42.9% and 42.8% in the Tulcea and Dâmbovița plots, respectively. Similar amounts of H were found in both roots and leaves for of Festuca arundinacea cultivated in the two experimental plots.

Results of the analysis of soil pH and electrical conductivity

Results of pH and electrical conductivity measurements of soil samples from the experimental plots in Dâmbovița and Tulcea are presented in Figure 4.

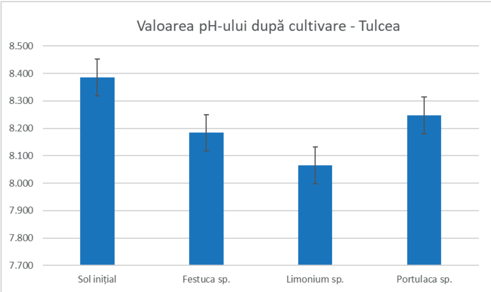

Figure 4. The pH and conductivity values of the soil samples before and after the cultivation of the species

According to the graph, it can be seen that in both experimental plots the pH value is modified after planting the three halophyte species, with the most notable changes occurring in the plots with *Festuca arundinacea*. These results are similar to those reported by Wang et al., 2021, who observed/ found that the cultivation of halophytes can reduce both soil pH and total salt content.

CONCLUSIONS

Sodium is the dominant element that influences the dynamics of minerals in the case of the experimental plot from Dâmbovița, where the soil salinity is significantly higher compared to the Tulcea plot. On the other hand, compared while the soil from Tulcea is clayey - rocky, the clayey-sandy soil structure from Dâmbovița Clayey-sandy soils allows a good preservation of moisture within the 10-30 cm soil depth range. The salt stress conditions in Dâmbovița induces a sodium dynamic exchange between the soil and the plants, with a significant decrease in soil salinity following cultivation of the *Limonium sinuatum.*

Overall, these results indicate a general trend in plants: higher concentration of the elements Na, Mg and Ca in the leaves compared to the roots, while the potassium concentration is slightly higher in the root.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development, Department of Statistics and also was financed from Project PN II Partnership No. 2365/2007.

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