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Population of *Halocnemum strobilaceum* (Pall.)M.Bieb in a dry salt lake of the Central Kyzylkum

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Abstract

Halocnemum strobilaceum (Pall.) M. Bieb. is a halophytic desert plant. This plant population is widespread in areas of high salinity of the world, including a Dry Salt Lake of the Central Kyzylkum. Analyzing the environment of the plant root system and the inorganic and organic composition of the soil of dried salt marshes using modern methods is of great importance in explaining the mechanisms of rapid growth and development of *H. strobilaceum* in areas affected by the stress factor. In the researches, with the help of IR spectroscopic and agrochemical methods, the amount of inorganic salts and organic substances in the soil around the root system of the *H. strobilaceum* plant was shown to be closer to the standard indicators than the amount of these substances in the soil of dry saline areas. The obtained results serve to explain the importance of *H. strobilaceum* and similar dominant species in the formation of plant populations in arid and saline areas.

Keywords

Groundwater, mineralization, precipitation, xerophytes, hyperhalophytes, IR spectra, functional groups, alkaline and alkaline earth elements, *Halocnemum strobilaceum*

Introduction

More than 833 million hectares of soils worldwide are already saline (1). In the Republic of Uzbekistan, in comparison with irrigated areas, the accumulation of salts in the soil was noted on significantly larger areas due to the natural salinization of agricultural areas. This was a result of an insufficient level of precipitation (2). For example, in the arid regions of the Central Kyzyl Kum of Uzbekistan, 100-150 mm of precipitation falls annually, while the observed evaporation of moisture from the soil surface significantly exceeds the total amount of precipitation (3). This process is mainly associated with high solar radiation, which, against the background of a minimum amount of precipitation, contributes to the intensive evaporation of moisture. As a result, a layer of salty white crust forms on the soil surface. This kind of excessive soil salinization in the Central Kyzyl Kum becomes the main limiting factor in the development of crop production and pasture animal husbandry, including karakul breeding (4).

Along with a high level of groundwater mineralization, soil salinization is caused by precipitation, which contributes to the dissolution of salts of easily soluble alkaline and alkaline earth elements. For example, the degree of groundwater mineralization in the Navoi-Kanimekh oasis varies from 2 to 10 g/L, in Bukhara from 1.6 to 10.8 g/L and in Karakul from 3 to 15 g/L. A higher degree of mineralization was noted in the fallow and empty peripheral areas of the Kagan and Karakul regions of the Bukhara region 20-50 g/L and more (5). In these areas, after intensive evaporation of water, the surface of the soil in the depressions and in the lowlands of the region is covered with salt crusts.

In most cases, the saline soils dominated by sulphate and chloride-sulfate types of mineralization, while on the periphery, where groundwater is under conditions of slow runoff, a variegated character of mineralization is observed; carbonate, chloride and sulphate types of salinization. It was previously noted that in the lower reaches of the Zarafshan River, a weak outflow of groundwater, their close occurrence to the soil surface and intensive evaporation of moisture enhance the accumulation of salts and contribute to the development of various variants of saline soils and solonchaks (6). Studies in 2012 and then in 2022 made it possible to assess the dynamics of vegetation cover development and changes in physicochemical parameters that caused salinization of the area we studied (7). With a parallel test of the influence of various gel-like compounds for the accumulation and preservation of moisture (8), as well as the participation of xerophytic and halophytic plants in the formation of sustainable plant community diversity in the Central Kyzyl Kum (9).

Salt soils are characterised by high osmotic pressure of the soil solution, the main cause of plant death, which exceeds the pressure of their cell sap. This factor reduces the level of water inflow into individual tissues, increases transpiration and impairs assimilation, respiration and the formation of sugars, ultimately leading to the drying and death of plants (4). In the process of evolution, the physiology of halophytes allowed them to adapt to growth and development in extreme conditions.

Halophytic plants vegetation is of great importance in the formation of secondary phytocenoses in saline areas. Halophytes are highly salinity tolerant plants: they usually grow and develop in environments with salt concentrations up to 5 g/L -1 (10). Halophytes play an important role in protecting ecosystems due to their regenerative abilities. Halophytic plants have various adaptations for growing in saline environments. These adaptations include production of suitable solutes to increase cytoplasmic osmotic pressure, accumulation of Na^+ in vacuoles and removal of Na^+ from cells. They are also characterized by the use of stimuli provided by endophytes and rhizosphere microorganisms (11).

It is appropriate to study the *H. strobilaceum* plant as a typical example in explaining the formation of plant phytocenoses in the saline areas of central Kyzylkum. *H. strobilaceum* is a hyperhalophyte shrub up to 60 centimeters tall, gray-green, spherical or cylindrical branches, non-smooth seeds. Widespread in Eastern Europe (from Italy and Greece to Ukraine) and Asia (Asia Minor, Caucasus, Iran, Iraq, Afghanistan, Pakistan, Arabian Peninsula, Kazakhstan, Siberia, Mongolia, Western China) (12). *H. strobilaceum* is a semi-shrub with elongated stems, dense, sometimes branching, mostly broad-rooted stems, growing in mounds. One-year-old branches are cylindrical, wet, cut into pieces. The leaves are not developed. According to one study, *H. strobilaceum* can be a promising species for phytoremediation due to its high adaptability to living in contaminated soil, stabilization and accumulation of metals in its tissues (13).

Based on the above analysis, the purpose of the study is to analyze the inorganic and organic composition of the plant root system environment and the soil of dried salt marshes using modern methods in order to explain the mechanisms of rapid growth and development of *H. strobilaceum* in areas affected by the stress factor.

Materials and Methods

The object of the study were soil samples, as well as the soil around the root system of the hyperhalophytic plant *Halocnemum strobilaceum* (Pall.), which grows on the site of a drying salt lake, located 150 km from the city of Navoi (Tamdy district, Navoi region, Republic of Uzbekistan) (Fig. 1). The *H. strobilaceum* plant is a shrub, or semi-shrub, belongs to the Amaranthaceae (Amaranthaceae) and

Fig. 1. Position of a drying up salt lake (40°49'59" N, 64°52'52" E, h=162, photo by Google Maps).

Halocnemum families, which, in the form of a narrow ribbon area on the alluvial lowland, grows along the coast of the Caspian Sea from Karabogazgol Bay to the city of Turkmenbashi (Krasnovodsk). A significant part of this plain is occupied by salt marshes and coastal depressions. The only halophytic representative of this kind of solonchaks is the knobby *H. strobilaceum*. Like almost all xerophytes and halophytes, sarsazan does not have developed leaves, is able to accumulate salts in its tissues, and tolerates extreme soil salinity. Therefore, *H. strobilaceum* is classified as a hyperhalophyte (14, 15).

Data from the IR spectrum analysis were used to study soil samples around the root system of *H. strobilaceum*, as well as soil around it at the site of the studied drying lake. The readings of the IR spectra were determined in the laboratory of the Department of Chemical Technology of the Navoi State State Technical University on an IR-Fourier spectrometer IK Tracer-100 (SHIMADZU) using the method of preparing pressed tablets in KBr. The absorption bands of infrared light correspond to antisymmetric wavelengths in the range from 400 to 4000 cm^{-1}. A comparison of the functional groups of elements was carried out according to the monograph (16).

In addition, we took into account the pH of the soil solution (based on the determination of H+ or OH-ions in the composition), its electrical conductivity (conductometric analysis of the amount of dissolved salts in the aqueous soil extract) and total alkalinity (%) arising under the influence of normal carbonates and bicarbonates. The dry residue, the proportion (in%) of soluble salts in the soil, the amount of chloride ion, and the amount of humus were also investigated (they were determined using the titrimetric method in the water extract) (17).

The amount of Na_2O and K_2O respectively, water-soluble sodium and potassium in the aqueous solution of the soil (in %), was determined on a flame photometer (18) and the amount of carbonates ($CaCO_3$, %) was determined by the volumetric gasimetric method on a calcimeter (19) according to the share of released CO_2. The amount of the corresponding ions in mg/kg was determined in the water extract of the soil using a spectrophotometer (wavelength for N-NH_4 440 nm; for P_2O_5 670 nm) (20, 21).

Results and Discussion

Analysis of the composition of salts in the soil of a drying lake showed the presence of three types of salinization in different parts of its territory - sulfate, chloride and carbonate, related to thenardite (Na_2SO_4), mirabilite (Na_2SO4 * $10H_2O$), halite (NaCl) and astrakhanite (Na_2SO_4 * $MgSO_4$ * $4H_2O$). In addition, the samples showed the presence of soluble forms of limestone ($CaCO_3$) in amounts that inhibit plant growth - more than 0.1-0.3%. The results obtained confirm the thesis about the easy solubility of these types of mineral salts, as well as the acceleration of the degree of their solubility under the action of root exudates of the sarsazan halophyte.

The application of the IR spectroscopic method of analysis allows one to get an idea of the morphology of different types of soil (the so-called "fingerprints"). In addition, the comparison of peaks in the diagrams makes it possible to localize the presence of different functional groups of chemical elements in the sample. To determine the localization sites of functional groups inherent in carbonates, we analyzed salts of chemically pure compounds of calcium carbonates ($CaCO_3$) and sodium carbonate (Na_2CO_3) (Fig. 2 A).

The place of localization of functional groups inherent in carbonates in the form of C-O in calcium carbonate was at wavelengths of 1300-1000 cm^{-1} and for C=O double bonds within wavelengths of 1700-1550 cm^{-1}. A very wide peak at wavelengths of 3800-3200 cm^{-1} is characteristic of the OH hydroxyl group. An analysis of the IR spectra of sodium carbonate (Na_2CO_3) showed a line that was not clearly pronounced, opaque to infrared rays, at the beginning of which, at wavelengths of 700-800 cm^{-1}, a convex peak was noted, apparently inherent in the characteristic lines of sodium functional groups.

The IR spectra of calcium ($CaSO_4$) and magnesium ($MgSO_4$) sulphates are shown in Fig. 2B. The characteristic peaks of the functional groups inherent in sulphate ions, intense absorption bands in the IR spectra are localized at 1000-1110 and 700-750 cm^{-1}, which belong to the sulphate ion. Most likely, these absorption peaks are formed as a result of reversible reactions of covalent sulphates with water and are present only in aqueous extracts (22-25).

The characterization of chemically pure compounds of magnesium chlorides ($MgCl_2$), iodized sodium (NaCl * I) and technical sodium (NaCl) showed the presence of characteristic lines inherent in the functional bonds of metals with –l-ions at different wavelengths. For example, for magnesium at wavelengths of 1500-1600 cm^{-1}, for iodinated sodium chloride an additional broad peak appeared at wavelengths of 1000-1250 cm^{-1} and an additional double and broad peak at wavelengths of 500-550 cm^{-1}, apparently inherent in iodine ions (Fig. 2C).

When identifying the IR spectra characteristic of chlorides, sulfates and carbonates, we used the atlas of the IR spectra of medicinal substances (20), since when soils are salinized, a characteristic feature is the dissolution of salts of alkaline and alkaline earth elements and their absorption by halophytes. To determine the IR spectra of dissolved chlorides, the frequency range (cm^{-1}), the intensity of the absorption bands of the functional groups of simple covalent bonds C-Cl are at wavelengths of 800-600 cm^{-1} with a strong stretching vibration. Sulfate bonds are characterized by wider intervals where average and simple covalent vibrations occur. For example, S-H are at wavelengths of 2600-2550 cm^{-1}, while variable stretching vibrations of SO functional groups are noted at wavelengths of 870-690 cm^{-1}. Double covalent bonds S=O in the form of sulfoxides are noted as strong stretching vibrations at wavelengths of 1225-980 cm^{-1}. Strong stretching vibrations of sulfones (SO_2) were noted at wavelengths of 1350-1300 cm^{-1} and 1160-1140 cm^{-1}. Strong stretching vibrations of the O–SO_2–bonds have already been noted at wave-

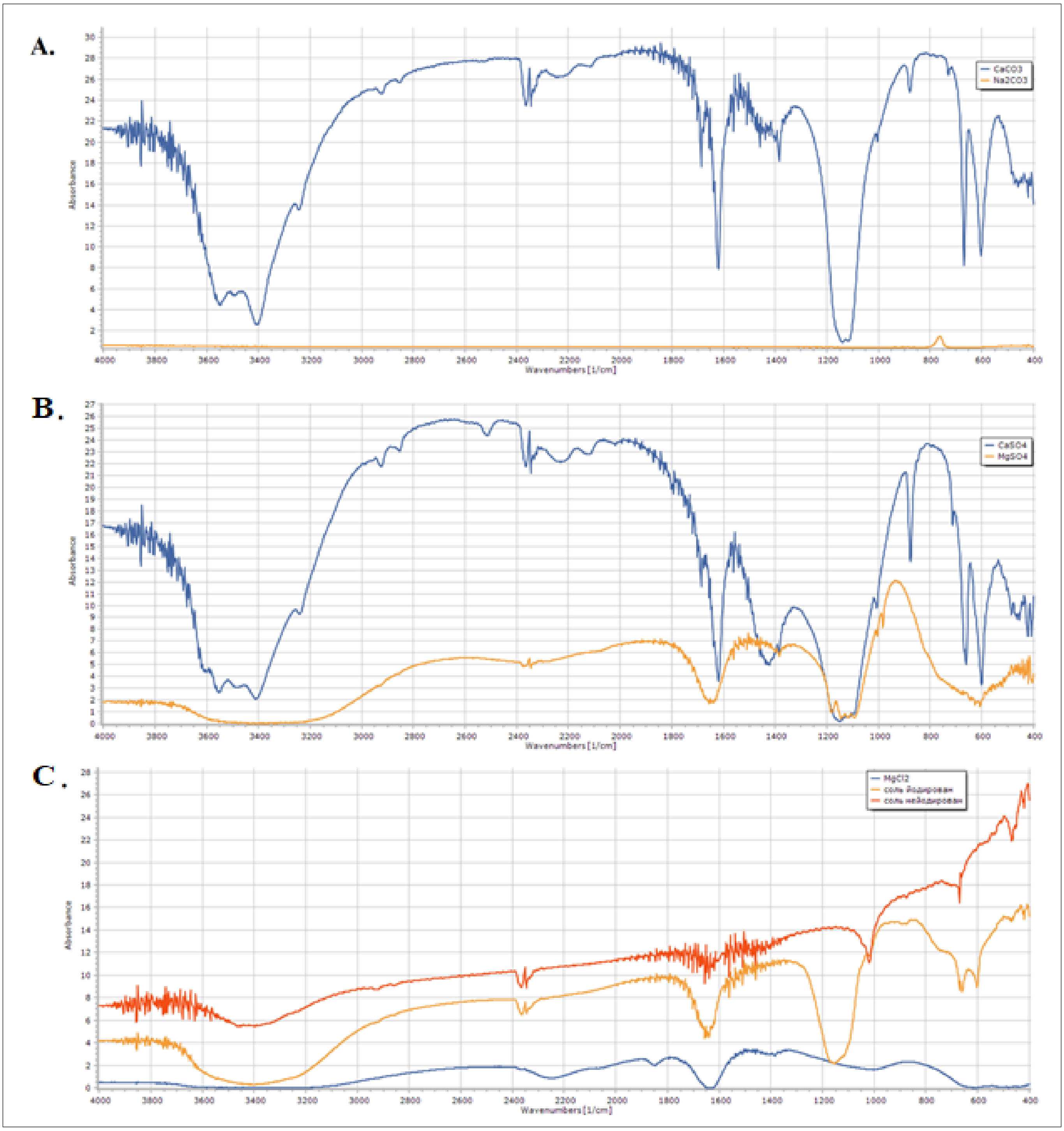

Fig. 2. IR spectra of some salts: **A** - calcium carbonates ($CaCO_3$) and sodium (Na_2CO_3); **B** - calcium sulfates ($CaSO_4$) and magnesium ($MgSO_4$); **C** - magnesium chloride ($MgCl_2$), iodized sodium (NaCl * I) and technical sodium (NaCl).

lengths of 1200-1145 cm^{-1} and 1420-1330 cm^{-1}. Intense absorption bands in the IR spectra at 1100-1110 and 620 cm^{-1} refer to the sulphate ion (22-25). Primary valence alcohols with wavelengths of 1075-1000 cm^{-1} and strong stretching vibrations can apparently serve as the basis for determining the IR spectra characteristic of C–O carbonates. C=O double bonds, which are characteristic of saturated aldehydes, ketones, carboxylic acids, and esters, are characterized by strong stretching vibrations within the wavelength range of 1750-1700 cm^{-1} (22). In the region of 880-840 cm^{-1}, carbonates are characterized by intense narrow absorption bands. The band at about 1100-1050 cm^{-1} is found in the spectra of rhombic carbonates and can serve as their diagnostic feature. However, the peak that appears in the region of 1000-1100 cm^{-1} can be due to both the absorption of carbonates and impurities, since silicates can also be strongly absorbed in this region (23).

Thus, a comparative characteristic of the IR spectra of salts of chlorides, sulfates and carbonates in undissolved and dissolved form showed a slight shift in the peaks of the location of functional groups due to stretching vibrations of varying degrees, however, in general, these peaks corresponded to the functional groups of these three types of compounds. Based on these comparative results, IR spectroscopic examinations of normal soil from a non-saline part of the desert, saline soil of a drying salt lake, and also from soil extracted around the root of the *H. strobilaceum* plant from this lake were carried out.

From the data of the curve line of the IR spectrum of normal soil (Fig. 3A), it can be seen that it is characterized by silicates with wide bands in the region of 1100-900 cm^{-1} with an absorption maximum of about 1030 cm^{-1}, which belongs to the Si-O stretching vibrations. The SiO_2 functional groups inherent in quartz, or silicon oxides, are part of more complex aluminosilicate minerals and cover the range of fluctuations from 1500 to 400 cm^{-1}. There are also 2 broad peaks at wavelengths of 870-1650 cm^{-1}, inherent in calcium carbonates, and the CO_3 functional group is concentrated in the frequency range of 1450-1430 and 875 cm^{-1}.

The hydroxyl group OH and water of crystallization, which are part of the particles, give absorption bands in the wavelength range of 3700-3300 cm^{-1} (stretching vibrations of the OH group) and in the frequency range of 1700-1600 cm^{-1} (deformation vibrations of H-O-H), which also appear in the indicated spectrum. Weak absorption bands in the wavelength range of 2400 and 2500 cm^{-1} are inherent in CH_2 hydrocarbon functional bonds, which indicate the presence of organic matter in the soil.

The IR spectra of the salty part of the soil of the drying lake, as well as the soil around the root part of the sarsazan, are shown in Fig. 3B and 3C respectively. The functional groups inherent in carbonates are especially clearly manifested in the form of a fine structure and intensity of absorption bands in the wavelength ranges of 892-872 and 758-700 cm^{-1}. The carbonate part with calcium functional groups is concentrated in the region of 729 and 711 cm^{-1}. Further study of the values of stretching vibrations 3 and 4, characteristic for $MgCO_3$ respectively, revealed that 1460 and 747 cm^{-1}, for nCO3-1443 and 745 cm^{-1}, for FeCO3-1433 and 735 cm^{-1}, for CaCO3-1418 and 708 cm^{-1}. The absorption peak due to crystallization water is noted in the regions of 3546-3448 and 1592-1534 cm^{-1}.

Thus, the position of the bands in the IR spectra can be used to judge the nature of isomorphic substitutions in the analyzed soil samples. Of particular interest is the accumulation of free and crystallized water around the root system of the plant, which is characterized by a broad peak in the wavelength range from 3000 to 3600 cm^{-1}. This circumstance confirms the idea that *H. strobilaceum* is superxerophytic. That is, the ability to survive in critical, stressful situations due to the storage of crystallized water around the root system of the plant. The stretching vibrations at these wavelengths are also very wide compared to the IR spectra of the saline part of the soil. The functional groups inherent in carbonates and chlorides, located at wavelengths from 1000 to 1700 cm^{-1}, are also strongly blurred around the root system, and the degree of solubility of carbonates and chlorides is confirmed by an increase in the transparency of the sample around the root system by infrared rays from 0 to 0.7%.

The nature of salinity indicates, perhaps, that after the retreat of the primitive ocean in the distant past, the area had a hilly character, consisting of the minerals limestone, dolomite, quartz etc., a dominant mixture of carbonates and then sulfates and then chlorides of solonchaks.

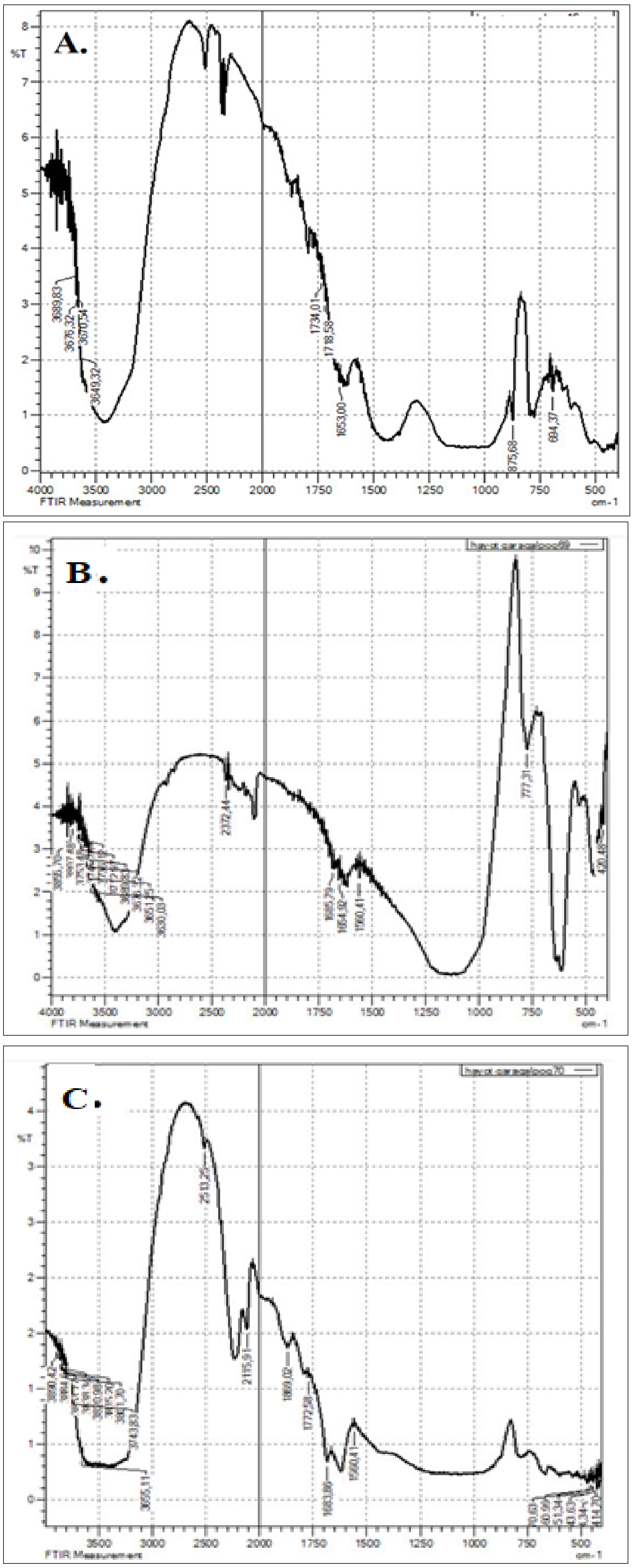

Fig. 3. IR spectrum of soil samples: (**A**) - non-saline soil of the Central Kyzylkum desert; salty part of soil (**B**) and soil sample (**C**) around the root system of a *H. strobilaceum* plant.

Gradually multiplying and settling, *H. strobilaceum* inhabits the salty space at the bottom of a drying lake (Fig. 4). On the prevailing heights of the hills, along the shore of the lake and farther from it, first sparse and then more dense thickets of various types of steppe plants

Fig. 4. Plants of the superhalophyte *H. strobilaceum* inhabiting the solonchak.

are noted. For example, the slopes of the depression around the lake are inhabited mainly by annual ephemera: *Roemuria orientalis, Arnebia baldshuanica, Ammothamnus lehmannii, Malcolmia grandiflora* and *Microcephala lamelata*. Then, as you move away from the lake, along with these ephemera, perennials appear, such as white wormwood, *Artemisia diffusa, Ceratoides ewersmanniana, Aellenia subaphylla* and *Salsola paletzkiana*. The only representatives of such phytocenosis are perennial shrubs - *Haloxylon aphyllum, Haloxylon persicum, Salsola arbuscula, Tamarix hispida* and *Ammodendron connoly*. Thus, in the composition of such phytocenosis, a regularity is noted: first, halophytes begin to grow on the salty part of the lake, and then, as we move away from the lake, a community of annual ephemera forms on the slopes. A little further from the shore, populations of perennial herbaceous plants form and mixed with them, single specimens of perennial shrubs.

As *H. strobilaceum* grows, a decrease in the area of the white coating of the salt crust is noted around the plant and the soil emerges (Fig. 4). Perhaps this is evidence of the ability of plants to dissolve and absorb salt, thereby purifying the soil from salt (the so-called "cultivation" of space). With a decrease in the amount of salt, space is freed up and the possibility of a gradual settlement of this place with other types of xerophytic plants is increased.

Analysis of the soil pH around the root part of the *H. strobilaceum* plant and the salty part of the soil showed (Table 1) that in the salty part of the soil it is 7.42, while around the root part of the plant it is 7.31, thereby approaching neutral for normal soil value.

Table 1. Chemical composition of soil samples around and near the root system of *H. strobilaceum* (sampling depth 0-70 cm)

Sign	Normal the soil	Saline soil	
		Soil around the root	Salt part of the soil
pH	6,5-7,5	7,42	7,31
Conductometer, µS/cm	<500	1160	1210
Dry residue, %	< 0,3	1,21	1,27
Total alkalinity, %	< 0,061	0,0321	0,0289
Chlorine ion, %	< 0,01	0,023	0,038
Na_2O %	0,023	0,011	0,015
K_2O %	0,01	0,0058	0,0044
$CaCO_{3\%}$	5-15	4,5	2,3

According to conductometric analysis, normally on non-saline soils, the amount of dissolved salts and their value is about 500 µS/cm, while in a sample from the salty part of the soil, the amount of salts reaches 1210 µS/cm and around the root part of the plant it decreases to 1160 µS/cm, which indicates the beginning of a gradual decrease in soil salinity. Similarly, around the root system of the plant, a gradual decrease in the dry residue of salts in quantitative terms from 1.27 to 1.21% was noted.

Similar processes were noted when analyzing the pH of the medium and the amount of dissolved salts of chlorine and sodium, due to the regular decrease in the amount of chlorine ions from 0.038 to 0.023% and sodium oxides from 0.015 to 0.011%. At the same time, there is a slight increase in the amount of potassium oxides from 0.0044 to 0.0058% and calcium from 2.3 to 4.5%. The presented results indicate the ability of the halophyte root system to dissolve not only chloride ions but also sodium, potassium and calcium salts, which enter the soil solution and gradually increase the total alkalinity from 0.0289 to 0.0321%. According to the degree of toxicity for plants, the most harmful are carbonates (Na_2CO_3, $NaHCO_3$), then chlorides ($NaCl$, $CaCl_2$, $MgCl_2$) and the least harmful are sulphates ($MgSO_4$, Na_2SO_4, $CaSO_4$).

Analysis of the content of the main nutrients (N, P, K) showed the following (Table 2). Around the root system of *H. strobilaceum*, the content of nitrogen in the form of ammonium ions was 3.11 mg/kg instead of 2.33 mg/kg, while the norm on ordinary soils is 31-45 mg/kg. The

Table 2. Main soil nutrients around and around the root system of *H. strobilaceum* (sampling depth 0-70 cm)

Sign	Normal the soil	Drying salt lake soil	
		Around the root	next to a plant
$N\text{-}NH_4$, mg/kg	31-45	3,11	2,32
P_2O_5, mg/kg	31-45	0,4	0,5
K_2O, mg/kg	201-300	152	92
Humus, %	0,8-1,2	0,46	0,41

amount of soluble phosphorus salts in the salt zone and around the root part of the plant was insignificant and amounted to 0.1 mg/kg. The amount of phosphorus compounds should normally be at least 41-45 mg/kg. There were quite a lot of potassium compounds around the root zone of plants—152 mg/kg, compared with the salty part, where the amount of potassium oxide was 92 mg/kg, while normally its amount should be 201 to 300 mg/kg.

As a rule, at high ambient temperatures and dry air, humus begins to decompose, while around the root system of the halophyte, its content in the soil begins to gradually increase, reaching 0.46%, while in the salty part of the soil it is 0.41%. Perhaps this is due to the activity of symbiont microorganisms (nitrogen-fixing microorganisms), which makes it possible to fix atmospheric nitrogen. It should be noted that the content of humus in the composition of desert and steppe soils normally ranges from 0.8 to 1.2%.

On highly saline soils, solonetzes and solonchaks, a new ecological niche is formed through gradual adaptation and colonization of these territories by hyperhalophytic plants - *Salicornia L., H. strobilaceum, H. belangeriana, K. foliatum, L. vulgare, F. laevis*, numerous saltwort's, annual haze and other plants. In the most extreme conditions for the existence of hyperhalophytes due to the high concentration of salt solutions in groundwater, solonchaks are considered. However, sparseness and species scarcity of vegetation cover are observed. An exception is *H. strobilaceum*. On the territory of the dried bottom of the studied lake, only the Sarsazan halophyte gradually spreads, which often forms extensive thickets on solonchaks, along saline shores of seas and lakes, and in solonchak depressions (26-28). This species is characterized by a wide distribution range, from the Mediterranean to the south of the European part of Russia, the Caucasus, the southern regions of Western Siberia and Central Asia.

Thus, an attempt to model the results obtained from a drying up salt lake in the Central Kyzyl Kum revealed the following. Primary salinization is associated with the preliminary dissolution of soil minerals consisting of easily soluble alkaline and alkaline earth elements, while secondary salinization is associated with natural processes. Among them are the weathering of minerals, the spread of salts by the wind, the washing away of salts dissolved by precipitation into the lowland, where, accumulating, they gradually form a solonchak. Thus, the flushing of dissolved salts into the depression led to their accumulation in a state critical for plant growth. An example is the results of the analyses of soil samples presented in this paper, which indicate the predominance of carbonate salinity over chloride and then sulfate. Under such extreme conditions, the only species of superhalophytic plants, *H. strobilaceum* has found its ecological niche. Growing in such conditions, the ability of sarsazan to store bound, crystallized water around its root system allows it to provide itself with moisture during the growing season. At the same time, by actively dissolving and assimilating salts of alkali and alkaline earth metals, the plant helps to reduce their amount in the soil solution, thus creating conditions for the colonization of this territory by other types of xerophytic plants. As you move away from the lake, conditions are formed on its slopes for the growth and development of communities of annual ephemera, then perennial herbaceous plants, with the inclusion of single specimens of perennial shrubs.

Thus, extreme environmental conditions led to a strong decrease in the amount of the main nutrient elements of the soil, the accumulation of salts of alkali and alkaline earth metals harmful to plants. Under such conditions, the superhalophyte sarsazan found its ecological niche, which is a precursor to the appearance of other species of steppe and desert plants in this area.

Conclusion

Data on the composition and amount of salts and nutrients in the soil around the roots of the plant *Halocnemum strobilaceum* (Pall.) M. Bieb, distributed in the dry saline lakes of Central Kyzylkum, showed that these plant populations are resistant to salinity stress. In the future, it is necessary to study the importance of these plant populations in the formation of secondary phytocoenoses of plants in areas with dry salinity.

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Authors contributions

BJ, BA, SHA and IA performed the experiments. BA and IT analyzed data. BJ, BA and SHA statistically analyzed results. IT, BA and ZI wrote the draft of the manuscript. IT conducted the critical revision of the manuscript. ZI worked out the concept and design, supervised and funded the experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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