The endemic halophyte *Sarcocornia carinata* Fuente, Rufo & Sánchez-Mata (Chenopodiaceae) in relation to environmental variables: elemental composition and biominerals

L Rufo[1*], MT Iglesias[1], V de la Fuente[2]

Addresses

1 Instituto de Investigaciones Biosanitarias, Facultad de Ciencias Experimentales, Universidad Francisco de Vitoria, E-28233, Pozuelo de Alarcón, Madrid, Spain

2 Facultad de Biología, Universidad Autónoma de Madrid, E-28049, Madrid, Spain

Corresponding author: l.rufo.prof@ufv.es; telephone number: +34917091400

Key words

Biominerals, halophyte, saline soils, *Sarcocornia*, succulence

Abstract

Aims: We propose a thorough study of the succulent halophyte *Sarcocornia carinata* endemic to the saline lagoons of the center of the Iberian Peninsula. We describe its elemental composition and possible seasonal variation in relation to edaphic and climatic variables, identify biominerals and analyze the distribution of salt ions and biominerals in tissue.

Methods: Plants and edaphic samples were collected in the four seasons of one year. Soils were analyzed for their pH, EC, color, and bioavailable concentration of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}. Soils and plants were analyzed for their total elemental and mineralogical composition. The distribution of elements and minerals in tissues was studied by scanning electron microscopy.

Results: Despite the variations observed in the edaphic and climatic variables, the variables studied in the plants varied slightly throughout the year. In the plants, Mg was the element that reflected climatic changes the most, while the K and Ca concentrations did not vary. Salty precipitates and crystallizations were distributed mainly in the epidermis, water storage parenchyma, cortex, and vascular vessels. Several crystals observed were compatible with halite, gypsum, glushinskite and weddellite.

Conclusions: The study corroborates that inland *S. carinata* behaves in the same way as other littoral succulent euhalophytes and reinforces the hypothesis that the concentration of elements and quantitative abundance pattern depend largely on the main adaptation mechanisms of halophytes.

Acknowledgements

This research was funded by the 2016 grant program of the Vicerrectorado de Investigación e Innovación de la Universidad Francisco de Vitoria. We would also like to express our gratitude to the personnel of the Servicio Interdepartamental de Investigación of the Universidad Autónoma de Madrid (SIDI-UAM, Spain) for their service. We would like to thanks to the three anonymous reviewers their valuable remarks and comments which contributed to the quality of this work.

Authors' contributions

MTI carried out the edaphic analysis. VF oversaw the sampling and plant analysis. LR originally planned the research but also carried out the plant analyses and led the writing. All the authors critically revised the manuscript.

Introduction

Soil salinization is considered one of the major threats to environmental sustainability. A considerable amount of agricultural soil worldwide is affected by salinity. Salt stress limits the growth and, therefore, the productivity of crops. Halophytes are flora that grow in saline soils and have been used as models to study different adaptations to salinity stress (Kamran et al. 2020). These plants are able to cope with high concentrations of salt using different strategies that involve osmotic adjustments and osmolyte synthesis to regulate oxidative stress, and anatomical, physiological and metabolic adaptations that enable salt avoidance by salt exclusion, salt secretion, shedding of salt-saturated tissues and organs, or succulence (Aslam et al. 2011; Flowers and Colmer 2008; Flowers et al. 2015).

As in other extreme environments (i.e. metalliferous soils, mine soils), the floristic composition of saline soils reflects the soil-plant relationship. The study of the elemental composition of flora in habitats with particular edaphic traits such as serpentine, other metalliferous soils or salt marshes, indicates that the specific vegetation found in these kinds of environments is related to the chemical composition of the soils (Brook 1998; Fuente et al. 2010). In particular, edaphic variables such as electrical conductivity, soil moisture, flooding period, soil texture, pH, Na^+ and Cl^- concentration and carbonate content have been related to the species' elemental composition and distribution, as well as the zonation patterns of vegetation from different saline enviroments (Donovan et al. 1997; Gil et al. 2014; Krüger and Peinemann 1996; Matinzadeh et al. 2013).

Research on the behavior of halophytes in their natural habitat could provide valuable information for understanding their adaptive mechanisms as well as their ecological significance. Climatic changes occur throughout the year and are closely related to the phenology of the plants (Kummerow 1983). Many of the studies on halophytes are based on analyses under laboratory-controlled conditions of specific variables such as ionic composition and cellular distribution, growth rates, the composition and content of osmolytes, antioxidant enzyme activity, etc. (Ben Hamed et al. 2014; García-Caparrós et al. 2017; Gil et al. 2011; Hameed et al. 2015; Ventura et al. 2014; Souid et al. 2016), but some authors also emphasize the importance of studying plants under natural conditions, based on the limited information available in this regard (Gil et al. 2014; Grigore et al. 2011).

Data obtained from field samples is usually variable and complex. However, some approximations have been carried out using halophytes with different ecologies and ranges of salt tolerance growing in littoral salt marshes and continental salt lagoons from California, Iran, and Spain. In these studies, ion concentrations in leaves and shoots, among other features, have been analyzed in relation to edaphic variables and seasonal changes (Donovan et al. 1997; Gil et al. 2014; Matinzadeh et al. 2013). The results seem to depend on the botanic origin of the species (dicotyledonous/monocotyledonous), adaptation mechanisms (succulent, salt accumulator/ion excluder through tissue/organ shedding or glands) and the ion considered (Na^+, K^+, Ca^{2+}, Mg^{2+}). More information about halophytes in the natural environment is needed for a better understanding of their adaptation mechanisms.

Sarcocornia carinata Fuente, Rufo & Sánchez-Mata is one of six species of the *Sarcocornia* genus identified growing in the Iberian Peninsula (Fuente et al. 2015). It is a succulent halophyte from the Chenopodiaceae family endemic to the center of Spain (Toledo, Ciudad Real). This plant forms almost monospecific masses that occupy temporarily flooded soils. Although *S. carinata* has a restricted distribution area, phylogenetic and cytological analyses indicate intraspecific genomic diversity (Fuente et al. 2013). The salt lagoons in the center of the Iberian Peninsula are important ecological reservoirs that sustain special flora and fauna. However, many of them are subject to great anthropic pressure of various forms (construction, desiccation, agriculture). Therefore, they are considered endangered areas and are under legal protection.

As with other *Sarcocornia* species, *S. carinata* has succulent and articulated photosynthetic stems that grow from woody stems. Succulence is associated with water storage and ion accumulation. The total element content has been calculated for several succulent halophytes such as *Sarcocornia pruinosa*, *S. ambigua*, *Arthrocnemum macrostachyum* or *Salicornia patula*. Sodium is the element that appears in the greatest concentrations, with values usually in the order of magnitude of 10^4 mg/kg dry weight (d.w.), but there are also high concentrations of K, Mg and Ca, although this varies slightly between species (Bertin et al. 2016; Fuente et al. 2010; Fuente et al. 2018). Therefore, high concentrations of these elements are expected.

Ion accumulation could lead to the formation of biominerals. Plants are known to produce biominerals in all their organs. Calcium biominerals such as oxalates are quite common; carbonates, sulfates and phosphates have also been observed in several plants, both halophytes and glycophytes (Weiner and Dove 2003). Other biominerals reported in plants are magnesium oxalates, silica, and iron in the form of jarosite and Fe-oxides (Monje and Baran 2005; Rodríguez et al. 2005). Fuente et al. (2018) have identified the chlorides halite and sylvite, and the oxalates glushinskite and weddellite in the succulent stem tissue of the littoral halophyte *Sarcocornia pruinosa*. However, there is a lack of information about the existence of seasonal changes in this process and the effect of soil composition on the biomineral composition of plants that adapt to salinity in the same way. As has been observed, tissue and cellular distribution patterns can also vary depending on the plant's adaptation to salinity (Pongrac et al. 2013). This could result in different biomineralization micropatterns among halophytes.

To provide new data about halophytes in their natural habitat, we propose a thorough study of *Sarcocornia carinata* over a year (2016-2017). The aims of this study are to describe the elemental composition of *S. carinata* and its possible seasonal variations in relation to edaphic and climatic variables, identify possible biominerals that form inside its tissues and analyze the tissue distribution of salt ions and biominerals.

Materials and Methods

Area of study

Two salt lagoons were chosen in which to carry out the study: Laguna Larga de Villacañas and Laguna de Peña Hueca, both in Toledo (Castilla La Mancha, Spain; Fig. 1). Both are in the SPA (special protection areas) and SCI (sites of community importance) wetlands of Castilla-La Mancha, protected by the European ecological network Natura 2000.

The Laguna Larga de Villacañas (39° 61' N; 3°32' W) is a shallow seasonal lagoon rich in chlorides that receives treated sewage waters from the water treatment plant of Villacañas village. This lagoon developed over quaternary deposits, and is surrounded by soils formed from limestone, marl, and clay. The Laguna de Peña Hueca (39°52' N; 3°33' W) is also a hypersaline seasonal lagoon rich in chloride and Mg^{2+}, formed by surface runoff over clays and tertiary materials. In the summer, it is usually completely dry (Cirujano 1980).

These territories have a Mediterranean pluviseasonal-oceanic bioclimate (Rivas-Martínez 2007) and the vegetation that grows there constitutes the halophilic geopermaseries of the center of the Iberian Peninsula, with *Suaeda braun-blanquetii*, *Puccinellia lagascana*, *Aleuropus littoralis* and *Lygeum spartum* (Rivas-Martínez 2011). In particular, the vegetation community where *S. carinata* is found is the phytosociological association *Puccinellio caespitosae-Sarcocornietum carinatae*. It covers a significant area of both lagoons where the terrain floods temporarily. The floristic composition of this community is almost monospecific, with *S. carinata* being the predominant species.

Fuente et al. (2013) analyzed specimens of *S. carinata* from both populations in their study on the phylogeny of the *Sarcocornia* genus in the Iberian Peninsula. Their results indicate a genetic variation between the specimens taken from the two lagoons selected for this study.

Plant phenology

Sarcocornia carinata Fuente, Rufo & Sánchez-Mata is a perennial succulent suffruticose chamaephyte composed of a basal woody stem from which grow succulent stems. A more extensive description is given in Fuente et al. (2013). During the spring, the plant begins to grow succulent stems. In summer, the plant is a green or reddish shrub with long succulent stems on which flowers begin to appear and develop throughout the season until the beginning of autumn. Seeds are usually mature at the end of autumn or beginning of winter. After maturation, the fertile stems dry and sometimes fall, so the plant remains mostly woody until the following spring.

Plant and soil sampling

To choose the sampling locations, a preliminary inspection was carried out analyzing the vegetation at both lagoons, especially the *S. carinata* community. Finally, three locations were selected in both areas based on the

surface area covered by the plant and the differences in humidity and soil texture reflected in the floristic composition of the community, as well as the color of the succulent stems: green or reddish (table 1).

A sample of a plant and soil was collected in each of the three locations at both lagoons in April, July and October of 2016 and February of 2017, completing the four seasons of a year. Therefore, 24 samples of plants and 24 samples of soil were analyzed. Specimens selected for collection in each sampling point were marked to harvest material always from the same individuals.

Plant samples were collected and cleaned with distilled water and then stored in a -80ºC freezer for subsequent analyses. Soil samples were air dried, sieved through a 2 mm sieve, and stored in plastic bags at room temperature for subsequent analyses.

Climatic Data

Climatic data were obtained from the SIAR (Sistema de Información Agroclimática para el Regadío) of the Spanish Ministerio de Agricultura, Pesca y Alimentación, accessible via http://eportal.mapama.gob.es/websiar/Inicio.aspx. Data were collected from the nearest weather station (La Puebla de Almoradiel, 20 km from Laguna Larga de Villacañas and 30 km from Peña Hueca). The data collected were mean temperature (T), maximum and minimum temperature (TMA, tma), precipitation (Pre) and evapotranspiration (EVT). The mean temperature and cumulative values for precipitation and evapotranspiration were calculated using data registered over a sixty-day period prior to each sampling date.

Edaphic analyses

Saturated soil-paste and physicochemical analysis

Saturated soil-pastes were prepared using 100 g of air-dried soil and distilled water, following a standard method (Rhoades 1982). These soil-pastes were left for 4 hours to reach an equilibrium and were then filtered in a vacuum with a Kitasato flask; each filtered soil extract was stored at 4ºC. A Crison CM 35+ conductivity meter was used to measure the electrical conductivity and a Crison 25 pH meter attached to a 50 50 T electrode to measure the pH of the filtered soil extract obtained from the saturated paste. The soil color was measured in both wet and dry soils using Munsell Soil Color Charts for each sample.

Bioavailable ion concentration in soil (IC)

The ion content of the soil solutions was analyzed using the ion chromatography technique (IC) and a Dionex DX600 model IC. The different ions were separated with two different analytical columns: anions (Cl^-, SO_4^{2-}, NO_3^-) were separated with a Dionex IonPac CS12A analytical column, Dionex IonPac CG12A guard column, Dionex DRS 600 suppressor and H_2SO_4 25mN eluent with a 1 mL/min flux; for cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+), separation was carried out with a Dionex IonPac AS9-HC analytical column, Dionex IonPac AGS9-HC guard column, Dionex AERS 500 suppressor and Na_2CO_3 9mM eluent with a 1 mL/min flux. NO_3^- and NH_4^+ concentrations were undetectable in most samples.

Elemental composition of the plants and soils (ICP-MS)

To quantify the total concentration of elements, all the plant samples were analyzed. As a reference of total elemental composition of soils, summer samples were used for this analysis.

Before the analysis, the succulent stems of *S. carinata* were separated, cleaned, dried, and powdered. 500 mg samples of plant powder were digested at high pressure using an 8 ml mixture of 65% HNO_3 and 2 ml mixture of 30% H_2O_2 in a Milestone MLS Ethos 1600 URM microwave digester, following the protocol described by Zuluaga et al. (2011). Soil samples were digested in a mixture of 3 ml HNO_3 and 1 ml HCl at 240 ºC.

Aliquots of the different plant and soil samples were analyzed by ICP-MS using an ELAN-6000 PE-Sciex (Toronto, Ontario, Canada) instrument for Na, Mg, K, Ca, Fe, Sr, Mn, Zn, Cu, Rb, Ba, Ni, Co, As and Pd concentration. Detection limits calculated by Zuluaga et al. (2011) are shown in table 2. Analyses were conducted in the Servicio Interdepartamental de Investigación of the Universidad Autónoma de Madrid (SIDI-UAM, Spain).

X-ray diffraction (XRD)

For the XRD analyses, two soil samples, on of each locality (summer samples from V3 and P3 sampling sites) were selected as representative samples of both areas. As microscopy analysis revealed similar composition and distribution in all analyzed sampled, for XRD analyses only two plant samples of different season were selected (spring and autumn samples from P3 site).

Soil samples were powdered in an agate mortar. For plants, succulent and woody stems were separated, cleaned, dried, and powdered using an IKA A11 basic instrument. Samples were analyzed using a X'Pert PRO Theta/2Theta (Almelo, Holland) analyzer with a graphite monochromator for Cu K-alpha-1 wavelength (1.5406 Å) and an X'Celerator fast detector. Identification was carried out using the HighScore Plus software created by Panalytical Plus and the ICDD PDF-4+ Full File database. Analyses and identification were conducted in the SIDI-UAM.

Scanning Electron Microscopy (SEM - EDX)

The plant samples collected every season from both lagoons were analyzed by SEM and an Energy Dispersive X-ray analyzer (EDX). In this study, we followed the methodology for the analysis of elements and localization of metals in plant material described by Rodríguez et al. (2005). The organs and tissues analyzed were woody and succulent stems (epidermis, parenchyma and cortex, central cylinder, and pith). Dry samples were cut into cross and longitudinal sections, these were then mounted onto conductive graphite stubs and sputters and coated in gold in a BIO-RAD SC 502 apparatus. The preparations were studied with a Hitachi S-3000N (Japan) SEM coupled with an INCAx-sight and Si-Li Detector (Oxford, England). An acceleration voltage of 20 kV and working distance of 15 mm were used in the analyses that were performed at room temperature.

Data and statistical analyses

To study the proportional relationship between the concentration of elements in plants and soils, two ratios were calculated: one related to the total concentration of elements in the soil (biological absorption coefficient) and the other related to the bioavailable concentration of elements in the soil solution (bioaccumulation factor).

The biological absorption coefficient (BAC) was calculated as follows:

$BAC = [P_i]/[S_i]$,

where $[P_i]$ is the total concentration of a named element (i) in plant succulent stems and $[S_i]$ is the total concentration of the same element in the corresponding soil. This ratio was calculated for Na, K, Mg, Cu, Zn, Mn, Ca, Rb, Sr, Fe, Co, Ni, Ba and Pb. As total concentration of soils was measured only for summer samples, this ratio has been calculated only for theses samples.

The bioaccumulation factor (BF) was calculated as follows:

$BF = [P_i]/[Sb_i]$,

where $[P_i]$ is the total concentration of a named element (i) in plant succulent stems and $[Sb_i]$ is the bioavailable concentration of the same element in the corresponding soil. This ratio was calculated for Na, K, Mg and Ca for all the samples.

For the statistical analyses, Statistical release 6.0 (Statsoft Inc., Tulsa, USA) software was used. Means, medians and standard deviations were calculated. The data were log transformed after being tested for normality with the Shapiro-Wilk test ($p > 0.05$). The difference between the two groups were calculated with a student's t-test ($p < 0.05$). The differences between several means were analyzed using a one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test ($p < 0.05$). A principal component analysis (PCA) was performed. Biplots were created from the components that explained most of the variability in the samples (PC1, PC2, PC3). The Pearson correlation coefficient (r) ($p < 0.05$) was used as an index of similarity.

Results

Seasonal changes in soil variables and their relationship with climatic data

Climatic seasonal oscillation throughout the year coincided with the Mediterranean bioclimate, which is characterized by two months of drought (Precipitation < 2*Temperature; Rivas Martínez 2007). Samples were collected in spring, summer and autumn of 2016, and winter of 2017. High temperatures were recorded in summer (TMA: 38.2 ºC) and autumn (TMA: 39.9 ºC). Temperatures lower than 0 °C were recorded in winter and spring. Spring was the most humid season (Prt: 98.3 mm) followed by autumn (table 3). A positive relationship was identified between the average temperature (T) and evapotranspiration (EVT), as well as the soil pH and electrical conductivity (EC). Compared to the previous ten years (table 3), temperature and evapotranspiration variables of the sampled period could be considered average, however, rainfall was over the average values.

The soil samples from Laguna Larga de Villacañas were more homogeneous than those of Laguna de Peña Hueca according to our field observations of their color and texture (table 1). An analysis of the color of the soil samples when dry and wet showed differences between the samples of both lagoons. Peña Hueca soils were a brown-reddish color in the wet samples and brownish-grey in the dry samples. Villacañas soils were a brown to brown-reddish color in the wet samples and brownish-grey in the dry samples.

The soils' pH varied from neutral to alkaline (6.8-8.4). Statistically significant differences were found between the seasonal average pH values of the water extracted from the saturated soil-paste of both sites. More alkaline values were observed in the autumn and summer samples, while the winter pH values were the most acidic. EC varied from 6.24 to 83.9 mS/cm. Statistically significant differences were only found in the Villacañas samples, where winter values were the lowest (7.83 mS/cm) and summer and autumn registered the highest salinity (table 4). This variation correlates with the type of climate and soil moisture in the sampling period. The EC values reveal the influence of the water table on salinity. In the Peña Hueca lagoon, we observed a variation in the EC according to the texture of the sample (sandy, silty, or clayey). The increase in EC could be attributed to a decrease in the coarse fractions of the soil that would limit internal drainage and thus prolong flooding with saline waters (Molina et al. 2001). The average EC in Peña Hueca soils values did not vary with the change of season.

The highest total concentrations in soil samples corresponded to Ca, Mg, Na, Fe and K, in that order and for both lagoons. Sr was present in concentrations of thousands of mg/kg, higher than any of the other elements analyzed (Mn, Ba, Rb, Zn, Cu, Pb, Ni, Co, As). Weak significant differences between average values were found only for Zn, Cu and Pb; the average concentration for each of these three elements was higher in the Villacañas samples (table 5).

When analyzing the bioavailable concentration of ions, Mg^{2+} and Na^{+} were present in the highest concentrations among the cations. The lowest values were found for K^{+}; Peña Hueca soils had the lowest bioavailable concentration of this element. Regarding the anions, SO_4^{2-} was present in higher concentrations than Cl^{-} in Villacañas soils, but both anions had similar average values in Peña Hueca soils (table 4).

Data from the soil samples from Peña Hueca were quite varied. This may be a consequence of the samples chosen for the study, which tried to cover all the different microhabitats where *S. carinata* grows in this lagoon (sandy to clayey soils, different degrees of humidity; table 1). Although no significant differences were found in any variable except pH (table 4), a positive correlation was observed between all the ions analyzed, except SO_4^{2-} and Ca^{2+}, and between the EC and all the ions, except Ca^{2+}.

Results from Villacañas were more homogeneous. In winter, these soils had less bioavailable concentrations than in the other seasons of all the ions analyzed except Ca^{2+}, which had a constant concentration throughout the year. Significant positive correlations were observed between all the ions analyzed, except Ca^{2+}, and pH, EC, and T. EVT was the only variable that had a positive relationship with all the ions.

Total concentrations of the elements in the plants

We did not find significant differences between the results of the samples from both lagoons, although most of the data were found to vary greatly. The average concentrations of the elements followed this order: Na > Mg, K > Ca > Fe > Sr > Mn > Zn > Cu, Rb, Ba, Ni > Co, Pb (table 6). The highest concentration in all the samples analyzed corresponded to Na, which varied from 36600 to 100951 mg/kg d.w. Concentrations of macronutrients were lower than those of Na. Such was the case of Mg (15558 to 41735 mg/kg d.w.), K (8340 to 24640 mg /kg d.w.) and Ca (2583 to 12179 mg /kg d.w.). Iron had the highest values among the micronutrients (70.1 to 612 mg

/kg d.w.). Sr (21.6 to 184 mg /kg d.w.) was the one of highest concentration among indifferent elements for plants studied (table 6).

There were statistically significant differences between monthly means for some elements (Na, Mg, Cu, Sr, Ni and Pb), but no clear pattern was observed except for Mg, which had the highest average concentration in the summer samples (30434 ± 6199 mg/kg d.w). Values of Na and Mg increased significantly from spring to summer. A significant positive correlation was observed for Ca, Mg and Sr concentrations. These elements also showed a negative correlation with Na. In addition, a negative relationship was observed between Mg and K, Cu and Zn concentrations.

K/Na ratios were between 0.14-0.73. No clear pattern was observed for K/Na ratios between seasons, although this ratio decreased significantly from spring to summer. Mg/Ca ratios varied between 1.68-6.86 and no significant differences were found between the average values of the different seasons.

*BAC and BF values *

Na, K and Mg were found in a higher concentration in the plant succulent tissues than in the total soil concentration. Na was present in the highest ratios. The rest of the elements had biological absorption coefficients (BAC) less than 1 (table 7).

Bioconcentration factors (BF) were calculated for Na, K, Ca, and Mg. The highest BF always corresponded to K. The others always had BFs higher than the unit, but the order of importance varied depending on the season and location (table 8). These values varied between seasons and significant differences were found in the Villacañas samples where winter values were the highest, except for Ca, which remained constant.

Principal component analysis (PCA) and correlations

A PCA was carried out to establish if there were statistically significant correlations between the seasonal variation of the total element concentration of the plants and the changes in the environmental and soil parameters analyzed. The biplots depicted show that PC1 and PC2 jointly explain 50% of the variance (Fig. 2). For PC1 (31%) Mg, Ni and Pb plant concentrations were found to be the variables with the greatest contribution to this component. Significant correlations were found between Mg and the average temperature (r = 0.448), precipitation (r = -0.631) and evapotranspiration (r = 0.668); Ni and the average temperature (r = -0.461), precipitation (r = 0.581) and evapotranspiration (r = -0.546); Pb and the average temperature (r = -0.765), precipitation (r = 0.426), evapotranspiration (r = -0.745), electrical conductivity (r = -0.513), pH (r = -0.61), soil Na^+ (r = -0.433), Mg^{2+} (r = -0.472) and Cl^- bioavailable concentrations (r = -0.528). The variables which most contributed to PC2 (20 %) were the concentrations of Sr, Ba and Ca in the plants. Significant correlations were obtained for Ba and the average temperature (r = -0.522) and pH (r = -0.511); Sr and the bioavailable concentration of Ca^{2+} in soil (r = 0.452).

Mineralogy

The soil samples analyzed showed the main mineral composition identified by XRD to be gypsum ($Ca(SO_4)_2 \cdot H_2O$), halite (NaCl), calcite ($CaCO_3$) and quartz (SiO_2).

In the plant samples, the XRD spectra varied between succulent and woody samples. The latter showed a higher proportion of amorphous material than the succulent stems. However, several peaks were clearly visible and allowed different minerals to be identified.

In all the plant samples analyzed, halite (NaCl), gypsum ($Ca(SO_4)_2 \cdot H_2O$), glushinskite ($Mg(C_2O_4) \cdot (H_2O)_2$ and weddellite ($Ca(C_2O_4) \cdot (H_2O)_2$ were identified to a greater or lesser extent. Halite was the most predominant mineral in succulent stems while there were only traces of oxalates. In the woody samples, although there was still a considerable proportion of halite, there were more oxalates and gypsum than in the succulent stems. Quartz (SiO_2) was also observed in woody stems and in one succulent sample, although only in traces (Fig. 3).

Distribution of elements in plant tissues

The anatomy of the cross sections of the succulent photosynthetic stems of *S. carinata* corresponded to that described for other species of this genus (Grigore and Toma 2017). The epidermis was composed of one layer of cells and multiple sunken stomata. It was followed by the palisade parenchyma and the water storage parenchyma. Among the parenchyma cells, tracheoid idioblasts could be seen, long spiral cells described in some articulated succulent Chenopodiaceae (Fig. 4a). The water storage parenchyma was composed of large, roundish cells with thin cell walls. This tissue made up most of the volume of the stem and included some vascular elements dispersed throughout this parenchyma (Fig. 4a). After this section was the stem with its central cylinder and pith (Fig. 4a). The central cylinder contained the vascular tissues (phloem and xylem) and parenchyma cells (Fig. 4b). The woody stems did not contain the palisade and water storage parenchyma but did have a cortex below the epidermis, followed by a wide central cylinder.

Salty precipitates and crystallizations were clearly visible inside the dry plant tissues throughout the year in both succulent and woody stems. The elemental composition, the relative proportion of the elements observed, and the micropattern of distribution remained constant in the plants throughout the year.

In succulent stems these salty precipitates were found in all the tissues but were more abundant in the water storage parenchyma and in the vascular tissues of the central cylinder. In the epidermis, a combination of Cl with Na and/or K were observed. The presence of large amorphous precipitates was very frequent in the water storage parenchyma (Fig. 5a-c; 6a-e). These were mainly composed of Na and Cl, but combinations of Cl and K, S and K, S and Ca or mixtures of Na, K, Mg, Ca, Cl and S were also frequent (Fig. 5d-g; 6c-g).

The cells of the water storage parenchyma closer to the central cylinder were usually full of multiple polyhedral crystals of Mg (Fig. 7b-d; 8b). These crystals took on different sizes and prismatic forms. Although amorphous combinations of S and Ca were quite common throughout the parenchyma, they were occasionally observed as long and fine polyhedral and raphides (Fig. 7f; 8d). The vascular vessels in the central cylinder were often found to be completely collapsed by salty crystallizations. These usually contained Na and Cl, and less abundant combinations of K with Cl or S, sometimes combined with Mg, Na and Ca, were also frequent (Fig. 5f).

Although salty crystals and precipitates were also observed in the woody stems, they were mostly confined to the cortex area, where they were abundant. Prismatic crystals and crystal aggregates of Ca or Mg and combinations of both were the most frequent (Fig. 7a, c, e; 8a). They were always observed in the large rectangular thin layer cells of the cortex, where they were present in most of the cells (Fig. 7a). Sometimes, even large-sized druses formed by the aggregation of multiple crystals were found (Fig. 7e). Additionally, on occasion, this tissue contained amorphous precipitates composed of Na, Mg, K, Cl and/or S; these were common but less frequent than Ca crystals. In the central cylinder, vascular vessels sometimes included vascular elements collapsed by amorphous precipitates of Na and Cl, or mixtures of the same elements observed in the rest of the tissues.

Discussion

The data gathered in this study were quite complex due to the quantity of variables and their heterogeneity. Most of the variables varied greatly, which is normal in field studies but also a reflection of the diversity of microhabitats where *S. carinata* grows. Although a higher number of samples would be preferred, they were limited because of the vulnerability of these habitats and this species, which has quite a restricted distribution and is endangered by anthropic actions. Nevertheless, we provide a complete study that includes climatic and edaphic parameters and a full characterization of the biominerals of this endemic halophyte.

Several studies show a variety of responses by the halophytes to the seasonal fluctuation of soil variables in relation to plant chemical composition. The soils analyzed in this study showed seasonal variations in the bioavailable concentration of ions, electrical conductivity and pH. These changes were clearly related to climatic variation, as has been observed in other saline areas (Gil et al. 2014).

Alhough the elemental composition of *S. carinata* was related to soil composition, it showed slight seasonal variation and for most of the elements analyzed, this variation did not seem to be related to electrical conductivity or the bioavailable concentration of ions. Similar results were obtained for other succulent halophytes of the same genus: for Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+} concentrations of *Sarcocornia fruticosa* and for Na^+ concentrations of *S. persica* subsp. *rudshurensis* (Gil et al. 2014; Matinzadeh et al. 2013). However, a correlation between seasonal

variation in plant composition and edaphic variables was observed for other succulent halophytes (*Halimocnemis pilifera, Atriplex verrucifera*, *Plantago crassifolia)* and non-succulent monocotyledonous halophytes (*Juncus maritimus*, *J. acutus*). This is sometimes explained by the phenological changes of the plants, such as leaf shedding during the reproduction phase of *H. pilifera* or trichome shedding during the growing season of *A. verrucifera* (Matinzadeh et al. 2013). These results indicate that variation in elemental composition and fluctuation throughout the year may depend greatly on the halophytes' main system of adaptation: succulence, shedding and/or exclusion.

Results obtained in this study confirm many of the adaptive strategies reported in the literature for dicotyledonous succulent halophytes. The anatomy of the stem sections was like other articulated succulent species, with the presence of a special water storage tissue that included big cells with large vacuoles filled with salt (Grigore et al. 2011; Fuente et al. 2018). Additionally, the quantitative abundance of the elements analyzed matched the general pattern described for halophytes (Chaudhary 2019), with Na being the main element in the total concentration, followed by Mg, K and Ca.

BF indexes confirmed the absorption of Na^+, K^+, Ca^{2+} and Mg^{2+}. Additionally, most of these elements (except Ca) had a higher total concentration in the plant than the corresponding total concentration in the soil. The maritime *Sarcocornia pruinosa*, growing in neutral-acid soils poor in K and Ca, showed similar results, except for Ca. BF ratios also indicated a preferential absorption of K^+ ions in all the seasons. Seasonal variation of BF ratios supports the fact that few differences were found between the seasonal average concentration of these elements in the plants, the lower bioavailable concentration in the soils (in winter), and the higher BF ratio. Therefore the plant maintains a similar total concentration throughout the year, independent of its phenological state.

Na concentrations of *Sarcocornia carinata* increased by the same order of magnitude as those reported in other succulent halophytes (i.e. 75160-47055 mg/kg d.w. for *S. pruinosa;* 52231 mg/kg d.w. for *Arthrocnemum macrostachyum*; 77185 mg/kg d.w. for *Salicornia patula*; 10190-24000 mg/kg d.w. for *S. ambigua* (Bertin et al. 2014; 2016; Fuente et al. 2010; 2018)). These values are remarkably high for vascular plants but could be considered normal for succulent halophytes (Brooks 1998). At a cellular level, most of the Na might be dissolved inside the vacuoles of epidermal cells, water storage parenchyma and vascular cells. This heterogeneous distribution was reported for several halophytes (Pongrac et al. 2013), where it was observed that some succulent halophytes did not accumulate Na in their photosynthetic tissues, probably to avoid toxicity. In *S. carinata*, this element has been observed as a common component of different salty precipitates and for the crystalized halite (NaCl), found in the aforementioned dry tissues. The distribution micropattern was the same as that reported for *S. pruinosa* (Fuente et al. 2018).

Seasonal differences among K concentrations in different dycotyledoneous and monocotyledoneous species have been reported, but no specific trend has been described (Gil et al. 2014; Matinzadeh et al. 2013). For *Sarcocornia carinata* K concentrations remain constant through the year. The mean concentrations obtained fall within the normal values for vascular plants, and are higher or similar to those reported for other halophytes (i.e. 7005-10137 mg/kg d.w. for *S. pruinosa;* 1810-24000 mg/kg d.w. for *S. ambigua* (Bertin et al. 2014; 2016; Fuente et al. 2018)). The K/Na ratios obtained were higher than those reported for *S. fruticosa*, *Inula crithmoides* and *Plantago crassifolia*, but lower than those obtained for some monocotylendons (Gil et al. 2014). As an essential macronutrient, it is not surprising to find this element in all the tissues of *S. carinata*. Additionally, K was found as a component of the precipitates of the vacuoles in the water storage parenchyma, together with Na, Mg and Cl, meaning the plant could also use K as other inorganic ion to mantain cell turgor. In contrast to *S. pruinosa*, we did not detect sylvite (KCl) by XRD.

In adition to Na, there were also high concentrations of Mg and the values obtained for *Sarcocornia carinata* were higher than the values reported for other succulent Chenopodiceae (i.e. 6357-6539 mg/kg d.w. for *S. pruinosa*; 77185 mg/kg d.w. for *Salicornia patula*; 4257 mg/kg d.w. for *Arthrocnemum macrostachyum*; 920-14000 mg/kg d.w. for *S. ambigua* (Bertin et al. 2014; 2016; Fuente et al. 2010; 2018)). The Mg concentration of *S. carinata* was the one that better reflected the climatic seasonal variation, showing a positive relationship with the average temperature and evapotranspiration, although no relationship was found with the edaphic parameters. On the other hand, Ca plant concentrations and bioavailable soil concentrations did not vary throughout the year. Calcium concentrations in *S. carinata* were similar to those found in other Chenopodiaceae, including other *Sarcocornia*

species. It has been suggested that Ca and Mg may have a protective role against the toxicity caused by Na (Grigore et al. 2012). Both elements can block K^+-efflux channels activated by the depolarization of the root plasma membrane and therefore avoid an excesive loss of K (Shabala and Pottosin 2014). Cases have been reported in which the toxicity caused by Mg^{2+} is higher than that caused by Na^+, such as in the germination of *Kalidium capsicum* (Tobe et al. 2002).

In any case, *Sarcocornia carinata* showed great tolerance to high Mg concentrations in its tissues. In the succulent stems at least, some of the Mg was inmobilized in the form of glushinskite and the rest could be dissolved inside the vacuoles, mainly in the water storage parenchyma, as was revealed by the XRD and microscopic analyses. Glushinskite has also been observed in *S. pruinosa* and in the Cactaceae *Opuntia ellisiana* (Fuente et al. 2018; Monje and Baran 2005).

Calcium, however, was more abundant inside the cortical cells of the woody stems, forming prismatic crystals compatible with weddellite, and it was observed to a lesser extent as long spicules compatible with gypsum, that could be form on drying. It is believed that Ca moves mainly through the xylem and its transport through the phloem is almost imperceptible (Hanger 1979). In dycotiledoneous plants Ca tends to be immobilized as Ca crystals in the bark of the stem, as seen in the woody stems of *S. carinata*. An XRD analysis confirmed the presence of calcium oxalate weddellite in both succulent and woody stems. Calcium oxalates are a common biomineral of plants and can form in all the organs. There are several functions atributted to these biominerals (as a defence against herbivores, calcium reserve or detoxification), but most of them remain controversial and need more proof (Sousa 2019; Karabourniotis et al. 2020).

Most of the rest of the elements were found to be inside the range of normal values for vascular plantas. However, Sr, which forms divalent cations, was found in remarkable concentrations, higher than those of other micronutrients such as Mn or Zn. Strontium is an insignificant element for plant metabolism but could lead to toxicity, despite the fact that it is commonly found in plants in its natural stable form. The physiological mechanisms for Sr uptake in plants seem to be related to its chemical similarity to other elements such as Ca. It is known that, in some cases, Sr enters the cell through Ca and K transporters, and it can move through the plant by xylem and phloem and be stored in plant tissues (Burger and Lichtscheidl 2019). It is also related to Ca biomineralization processes (Franceschi and Schueren 1986). We did not detect this element by EDX in the various calcium crystal or saline precipitates observed in *S. carinata*, although it could be present in concentrations under the detection limit of the techniques used in this study.

Conclusions

The results obtained after this thorough study of *Sarcocornia carinata* match previous information about other succulent euhalophytes from littoral salt marshes. Despite the phenological changes and the dramatic variations observed in the edaphic and climatic variables, the total concentration of elements, quantitative abundance pattern, elemental distribution micropattern, and biominerals and their localization were almost the same for the entire year. The main element in the plant that reflected the climatic changes was Mg, while the K and Ca concentration in the plant remained stable throughout the different seasons and phenological changes. This may indicate that these features depend largely on the halophyte's main adaptation mechanisms and, at least with regard to the variables studied in this work, the behavior of succulent halophytes should be different from salt excluders.

Besides salt accumulation in vacuoles, we can confirm that succulent halophytes can form biominerals in their tissues. It seems that halite (NaCl), glushinskite ($Mg(C_2O_4)\cdot(H_2O)_2$) and weddellite ($Ca(C_2O_4)\cdot(H_2O)_2$) are common biominerals in succulent halophytes. Although these minerals are present in succulent and woody stems, Mg and Ca oxalates have a more specific distribution and most often accumulate in specific tissues in both kind of stems.

Despite the number of studies on halophyte plants, there is still a need for further information regarding the management of some potentially toxic elements that could be easily absorbed and accumulated by these types of plants, in addition to biomineralization processes and differences between halophytes that use other strategies.

References

Aslam R, Bostan N, Amen N, Maria M, Safdar W (2011) A critical review on halophytes: salt tolerant plants. J Med Plants Res 5(33): 7108-7118

Bautista I, Boscaiu MT, Lindón A, Llinares JV, Lull C, Donat-Torres MP, Mayoral O (2016) Environmentally induced changes in antioxidant phenolic compounds levels in wild plants. Acta Physiol Plant 38(9): 1-15 10.1007/s11738-015-2025-2

Ben Hamed K, Chibani F, Abdelly C, Magne C (2014) Growth, sodium uptake and antioxidant responses of coastal plants differing in their ecological status under increasing salinity. Biologia 69(2): 193-201

Bertin RL, Gonzaga LV, Borges GSC, Azevedo MS, Maltez HF, Heller M, Micke GA, Ballod LB, Fett R (2014) Nutrient composition and identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC-EIS-MS/MS. Food Res Int 55: 404-411

Bertin RL, Maltez HF, Gois J, Borges DLG, Cmapelo G, Gonzaga LV, Fett R (2016) Mineral composition and bioaccessibility in *Sarcocornia ambigua* using ICP-MS. J Food Compos Anal 47: 45-51

Brooks RR (1998) Plants that hyperaccumulate heavy metals. CAB International, Cambridge

Burger A, Lichtscheidl I (2019) Strontium in the environment: Review about reactions of plants towards. Sci Total Environ 653: 1458-1512

Chaudhary D (2019) Ion accumulation pattern of halophytes. In: Hasanuzzaman M, Shabala S, Fujiita M (eds) Halophytes and climate change: adaptative mechanisms and potential uses. CAB International, Pondicherry, India, pp 137-151

Cirujano S (1980) Las lagunas manchegas y su vegetación I. Anal Jardín Bot Mad 37 (1): 155-191

Donovan L, Richards J, Schaber E (1997) Nutrient relations of the halophytic shrub, *Sarcobatus vermiculatus*, along a soil salinity gradient. Plant Soil 190: 105-117

Flowers T, Colmer T (2008) Salinity tolerance in halophytes. New Phytol 179: 945-963

Flowers T, Munns R, Colmer T (2015) Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann Bot-London 115: 419-431

Franceschi V, Schueren A (1986) Incorporation of strontium into plant calcium oxalate crystals. Protoplasma 130: 199-205

Fuente V, Oggerin M, Rufo L, Rodríguez N, Ortuñez E, Sánchez-Mata D, Amils R (2013) A micromorphological and phylogenetic study of *Sarcocornia* A.J. Scott (Chenopodiaceae) on the Iberian Peninsula. Plant Biosyst 147: 158-173

Fuente V, Rufo L, Rodríguez N, Amils R, Zuluaga J (2010) Metal accumulation screening on the Río Tinto flora (Huelva, Spain). Biol Trace Elem Res 134: 318-341

Fuente V, Rufo L, Rodríguez N, Sánchez-Mata D, Franco A, Amils R (2015) A study of *Sarcocornia* A.J. Scott (Chenopodiaceae) from Western Mediterranean Europe. 150(2): 343-356

Fuente V, Rufo L, Sánchez-Gavilán I, Ramírez E, Rodríguez N, Amils R (2018) Plant tissues and embryos biominerals in *Sarcocornia pruinosa*, a halophyte from the Río Tinto salt marshes. Minerals 8(11) https://doi.org/10.3390/min8110505

García-Caparrós P, Llanderal A, Pestana M, Correira PJ, Lao MT (2017) Nutritional and physiological responses of the dicotyledonous halophyte *Sarocornia fruticosa* to salinity. Aust J Bot 65(7): 573-581

Gil R, Bautista I, Boscaiu M, Lindón A, Wankhade S, Sánchez H, Llinares J, Vicente O (2014) Responses of five mediterranean halophytes to seasonal changes in environmental conditions. AOB Plants 6, plu049 doi:10.1093/aobpla/plu049.

Gil R, Lull C, Boscaiu M, Bautista I, Lidón A, Vicente O (2011) Soluble carbohydrates as osmolytes in several halophytes from a mediterranean salt marsh. Not Bot Horti Agrobo 39(2): 9-17

Grigore M, Boscaiu M, Llinares J, Vicente O (2012) Mitigation of salt stress-induced inhibition of *Plantago crassifolia* reproductive development by supplemental calcium or magnesium. Not Bot Horti Agrobo 40: 58-66

Grigore M, Boscaiu M, Vicente O (2011) Assessment of the relevance of osmolyte biosynthesis for salt tolerance of halophytes under natural conditions. Eur J Plant Sci Biotechnol 5: 12-49.

Grigore M, Toma C (2017) Anatomical adaptations of halophytes: a review of classic literature and recent findings. Springer International Publishing, Switzerland

Hameed A, Gulzar S, Aziz I, Hussain T, Gul B, Khan MA (2015) Effects of salinity and ascorbic acid on growth, water status and antioxidant system in a perennial halophyte. AOB Plants 7(pvl004) doi: 10.1093/aobplan/pvl004.

Hanger B (1979) The movement of calcium in plants. Commun Soil Sci Plant 10(1-2): 171-193

Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattja MS, Saleem MH, Adil M, Heidari P, Chen J (2020) An overview of hazardous impacts of soil salinity in crops, tolerance, mechanisms, and amelioration through Selenium supplementation. Int J Mol Sci 21 (1): 148

Karabourniotis G, Horner HT, Bresta P, Nikolopoulos D, Liakopoulos G (2020) New insights into the functions of carbon-calcium inclusions in plants New Phytol https://doi.org/10.1111/nph.16763

Krüger H, Peinemann N (1996) Coastal plain halophytes and their relation to soil ionic composition. Plant Ecol 122 (2): 143-150

Kummerow J (1983) Comparative Phenology of Mediterranean-Type Plant Communities. In: Kruger F.J., Mitchell D.T., Jarvis J.U.M. (eds) Mediterranean-Type Ecosystems. Ecological Studies (Analysis and Synthesis), vol 43. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-68935-2_17

Matinzadeh Z, Breckle S, Mirmassoumi M, Akhani H (2013) Ionic relationships in some halophytic Iranian Chenopodiaceae and their rhizospheres. Plant Soil 372: 523-539

Molina J, Pertiñez C, de la Cruz M (2001) Datos sobre la relación suelo-vegetación en los saladares de Cordobilla (Albacete, España). Revista de Estudios Albacetenses 1 (1): 217-232

Monje P, Baran E (2005) Evidence of formation of glushinskite as a biomineral in Cactaceae species. Phytochemistry: 66: 611-614

Pongrac P, Vogel-Mikus K, Regbar M, Kaligaric M, Vavpetic P, Kelemen M, Grlj N, Shelef O, Golan-Goldhirsh A, Rachmilevitch S, Pelicon P (2013) On the distribution and evaluation of Na, Mg and Cl in leaves of selected halophytes. Nucl Instrum Meth B 306: 144-159

Rhoades J (1982) Soluble salts. In: Page A (ed) Methods of soil analysis part 2. Madison, WI, USA: Agronomy monograph nº9, American Society of Agronomy, pp 167-179

Rivas-Martínez S (2007) Mapa de series, geoseries y geopermaseries de vegetación de España [Memoria del mapa de vegetación potencial de España]. Parte I. Itinera Geobot 17: 5-436

Rivas-Martínez S (2011) Mapa de series, geoseries y geopermaseries de vegetación de España [Memoria del mapa de vegetación potencial de España]. Parte II. Itinera Geobot18(1): 5-800.

Rodríguez N, Menéndez N, Tornero J, Amils R, Fuente V (2005) Internal iron biomineralization in *Imperata cylindrica*, a perennial grass: chemical composition, speciation and plant localization. New Phytol 165: 781-789

Shabala S, Pottosin I (2014) Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. Physiol Plantarum 151: 257-279

Souid A, Gabriele M, Longo V, Pucci L, Bellani L, Smaoui A, Abdelly C, Ben Hamed K (2016) Salt tolerance of the halophyte *Limonium delicatulum* is more associated with antioxidant enzyme activities than phenolic compounds. Funct Plant Biol 43(7): 607-619

Sousa E (2019) Are calcium oxalate crystals a dynamic calcium store in plants?. New Phytol 223: 1707-1711

Tobe K, Li X, Omasa K (2002) Effect of sodium magnesium and calcium salts on seed germination and radicle survival of a halophyte, *Kalidium caspicum* (Chenopodiaceae). Aust J Bot 50: 163-169

Ventura Y, Myrzabayeva M, Alikulov Z, Omarov R, Khozin-Goldberg I (2014) Effects of salinity on flowering, morphology, biomass accumulation and leaf metabolites in an edible halophyte. AOB Plants, 6: plu053 doi:10.1093/aobpla/plu053.

Weiner S, Dove P (2003) An Overview of Biomineralization Processes. Revi Mineral Geochem 24(1): 1-29

Zuluaga J, Rodríguez N, Rivas-Ramírez I, Fuente V, Rufo L, Amils R (2011) An improved semiquantitative method for elemental analysis of plants using inductive coupled plasma mass spectrometry. Biol Trace Elem Res 144: 1302-1317

Table 1: Information about the sample locations. Latitude and longitude coordinates, conditions of the plant community and specific details about the plants collected and the ecology and soil appearance.

Location	Sample site	Coordinates	Plant community	Ecology
Laguan Larga de Villacañas	V1	39.613522 - 3.327143	Dense coverage of *S. carinata.* Plants with green succulent stems, affected by grazing.	Areas temporarily flooded with occasional presence of algae. Brownish silty soil.
	V2	39.612758 - 3.326767	Scarce coverage of *S. carinata.* Plants with green succulent stems, affected by grazing.	Areas temporarily flooded. Around edges of puddles that lack vegetation. Brownish silty soil.
	V3	39.611884 - 3.326531	Dense coverage of *S. carinata.* Plants with reddish succulent stems, affected by grazing.	Areas temporarily flooded with occasional presence of algae. Brownish silty soil.
Laguna de Peña Hueca	P1	39.520184 - 3.335273	Scarce coverage of *S. carinata.* Plants with green succulent stems, affected by grazing.	Dry area rarely flooded. Sandy soil.
	P2	39.520395 - 3.335691	Dense coverage of *S. carinata.* Plants with green succulent stems, affected by grazing.	Areas temporarily flooded with occasional presence of algae. Brownish silty soil.
	P3	39.520184 - 3.336928	Dense coverage of *S. carinata.* Plants with reddish succulent stems, affected by grazing.	Humid area temporarily flooded. Around the edges of the lagoon. Reddish sticky and clayey soils.

557 **Table 2:** Detection limits for ICP-MS Elan 6000 instrument described in Zuluaga et al. (2011)

Element	Detection limit (µg/l)
As	0.59
Ba	0.06
Ca	1.81
Co	0.02
Cu	0.21
Fe	8.26
K	2.56
Mg	0.12
Mn	0.09
Na	1.08
Ni	0.16
Pb	0.02
Rb	0.009
Sr	0.03
Zn	0.26

558

559

560

Table 3: Values of the climatic variables analyzed. Historical annual values from 2006 to 2017 and seasonal values of the period of sampling (spring, summer and autumn of 2016, and winter of 2017). Climatic variables: T: average temperature (ºC); TMA: maximum absolute temperature (ºC); tma: minimum absolute temperature (ºC); Prt: accumulative precipitation (mm); EVT: accumulative evapotranspiration (mm). 95% CI: 95% confidence interval of the mean from 2006 to 2017. Seasonal data are expressed as follow: mean ± standard deviation of T values, accumulative values of P and EVT and absolute values of TMA and tma.

		T	TMA	tma	Prt	EVT
Historical annual values	2006	14.2	38.1	-8.2	384.8	1223.3
	2007	12.8	38.5	-10.2	365	1128.3
	2008	13.1	38.9	-9	364.2	1111.3
	2009	14.3	38.7	-13.3	284.2	1234.4
	2010	13.3	39.1	-9.1	537.8	1117.4
	2011	14.2	37.6	-8.9	264.5	1173.3
	2012	13.6	42.1	-10.2	317	1165.9
	2013	13.3	38.5	-8.1	387	1060.4
	2014	14.5	37.3	-8.6	276.5	1122.3
	2015	14.5	40.7	-7.9	202.2	1194
	2016	14.2	39.9	-7.3	413.7	1122
	2017	14.6	42.9	-9.4	203	1189
	95% CI	13.5 - 14.3	38.3 - 40.4	-10.2 - (-8.2)	274 - 393	1122 - 1185
Seasonal values	Spring 2016	7.78 ± 2.66	24.6	-7.3	98.3	144
	Summer 2016	21.1 ± 4.44	38.2	3.9	7.6	334
	Autumn 2016	17.5 ± 4.17	39.9	0.1	53.3	152
	Winter 2017	4.44 ± 2.91	17.6	-9.4	32.7	53.2

Table 4: Values of the edaphic variables analyzed. Edaphic variables: pH; EC: electrical conductivity (ms/cm); bioavailable concentration of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-} (mg/ kg d.w.). Data are expressed as mean ± standard deviation. Statistically significant differences between means are indicated as ** $p < 0.01$; *** $p < 0.001$. Different lower-case letters indicate statistically significant differences. Values in bold indicate the highest values for a given variable (in a row) and those highlighted in gray are the lowest values for a given variable (in a row).

	Spring	**Summer**	**Autumn**	**Winter**
		Peña Hueca		
pH***	7.45 ± 0.04^{a}	7.85 ± 0.24^{ab}	8.27 ± 0.09^{b}	6.95 ± 0.17^{c}
EC (mS/cm)	28.9 ± 6.3	56.9 ± 36.0	26.1 ± 6.32	13.5 ± 5.25
Mg^{2+} (mg/kg)	2199 ± 368	8543 ± 7403	1815 ± 366	728 ± 196
Na^{+} (mg/kg)	2040 ± 719	6135 ± 4956	2087 ± 740	772 ± 360
Ca^{2+} (mg/kg)	445 ± 132	549 ± 70	531 ± 113	379 ± 67
K^{+} (mg/kg)	139 ± 37	240 ± 139	138 ± 65	72.3 ± 9.5
SO_4^{2-} (mg/kg)	5696 ± 609	19080 ± 16876	5145 ± 871	2647 ± 346
Cl^{-} (mg/kg)	5787 ± 1644	19274 ± 15767	6209 ± 1594	2488 ± 1342
		Villacañas		
pH***	7.41 ± 0.04^{a}	7.60 ± 0.18^{b}	**8.14 ± 0.20^{c}**	7.11 ± 0.09^{a}
EC (mS/cm)***	41.2 ± 2.98^{a}	70.3 ± 5.9^{b}	50.4 ± 5.29^{ab}	7.83 ± 1.39^{c}
Mg^{2+} (mg/kg)***	4547 ± 668^{ab}	10803 ± 2766^{a}	8386 ± 2608^{ab}	470 ± 134^{c}
Na^{+} (mg/kg)***	4503 ± 197^{a}	8595 ± 2627^{a}	7649 ± 1489^{a}	469 ± 174^{b}
Ca^{2+} (mg/kg)	603 ± 132	625 ± 221	538 ± 240	372 ± 7
K^{+} (mg/kg)***	529 ± 14^{a}	923 ± 382^{a}	758 ± 134^{a}	111 ± 23^{b}
SO_4^{2-} (mg/kg)**	14769 ± 1951^{a}	28439 ± 7953^{a}	30580 ± 11328^{a}	3071 ± 538^{b}
Cl^{-} (mg/kg)***	9854 ± 1710^{a}	18411 ± 6061^{a}	18122 ± 3674^{a}	875 ± 433^{b}

575 **Table 5:** Total concentration of elements in summer soil samples from Peña Hueca and Villacañas. Soils have
576 been analyzed by ICP-MS and data are expressed as mg/kg. Me: median; M ± SD: mean ± standard deviation. N
577 = 3. Statistically significant differences between means are indicated as * $p > 0.05$. Values in bold indicate that
578 this is the highest value for a given variable (in a row).

Location	**Peña Hueca**		**Villacañas**	
	Me	M ± SD	Me	M ± SD
Ca	116708	122090 ± 28701	163375	160287 ± 13552
Mg	33368	39649 ± 16408	30027	28398 ± 4253
Na	10042	7992 ± 6035	14397	13775 ± 3137
Fe	7815	9982 ± 4845	12473	12862 ± 1059
K	5799	7204 ± 3074	9916	10171 ± 809
Sr	1923	1876 ± 258	1690	1620 ± 186
Mn	181	204 ± 66.4	227.3	236 ± 28.3
Ba	174	172 ± 12.6	176	178 ± 14.0
Rb	30.6	38.6 ± 18.5	48.0	47.8 ± 3.01
Zn*	15.7	18.5 ± 10.3	41.2	**46.6 ± 13.4**
Cu*	5.36	6.56 ± 3.31	19.16	**22.1 ± 8.26**
Pb*	7.34	8.88 ± 2.95	19.3	**19.7 ± 2.28**
Ni	7.76	9.66 ± 4.58	13.7	14.0 ± 0.60
Co	2.91	3.65 ± 1.37	5.54	5.46 ± 0.25
As	3.95	4.95 ± 1.83	4.88	4.88 ± 0.74

579

580

Table 6: Total concentration of elements in the succulent stems of *S. carinata* analyzed by ICP-MS and expressed in mg/kg dry weight and Na/K and Mg/Ca ratios. Data expressed as mean ± standard deviation. n=6. Statistically significant differences between means are indicated as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different lower-case letters indicate statistically significant differences. Values in bold indicate the highest values for a given variable (in a row).

Season	**Spring**	**Summer**	**Autumn**	**Winter**
Phenology	Mainly woody stems. Sprouts from new succulent stems	Succulent stems fully grown. Beginning of the flowering period	Succulent stems fully grown, mature flowers. Beginning of the fruition period	Mainly woody stems. Dry spikes, short succulent stems
Na* **	50025 ± 10828^{a}	80001 ± 15837^{b}	68395 ± 13236^{ab}	80111 ± 1380^{b}
Mg* **	20612 ± 2419^{a}	**30434 ± 6199^{b}**	17677 ± 1836^{a}	17914 ± 2511^{a}
K	19517 ± 6233	15422 ± 4648	19010 ± 3275	20147 ± 4240
Ca	8962 ± 1790	7906 ± 2076	6887 ± 2776	7186 ± 2662
Fe	294 ± 179	138 ± 81	144 ± 58	240 ± 104
Sr*	87.2 ± 22.1^{ab}	103.9 ± 48.2^{a}	49.5 ± 20.9^{b}	57.2 ± 34.6^{ab}
Mn	30.6 ± 9.1	33.9 ± 14.9	41.9 ± 11.6	45.4 ± 14.0
Zn	18.6 ± 6.7	11.0 ± 7.05	15.33 ± 4.15	16.09 ± 8.63
Cu **	5.40 ± 2.17^{a}	4.80 ± 0.56^{a}	9.43 ± 2.70^{b}	6.09 ± 2.02^{ab}
Rb	4.26 ± 1.25	3.19 ± 0.82	3.21 ± 0.58	2.91 ± 0.44
Ba	3.48 ± 2.06	1.69 ± 0.90	1.53 ± 0.82	3.38 ± 2.41
Ni*	1.15 ± 0.44^{ab}	0.55 ± 0.25^{a}	1.12 ± 0.54^{ab}	1.27 ± 0.56^{b}
Co	0.15 ± 0.05	0.12 ± 0.06	0.11 ± 0.05	0.24 ± 0.16
Pb* **	0.39 ± 0.29^{a}	0.11 ± 0.11^{b}	0.19 ± 0.07^{ab}	0.62 ± 0.25^{a}
K/Na **	0.41 ± 0.18^{a}	0.19 ± 0.06^{b}	0.28 ± 0.06^{ab}	0.25 ± 0.05^{ab}
Mg/Ca	2.38 ± 0.58	4.11 ± 1.35	3.14 ± 1.91	2.77 ± 1.02

589 **Table 7:** Biological absorption coefficient (BAC) of summer samples. Data expressed as median (Me) and mean
590 ± standard deviation (M ± SD). n=6. Statistically significant differences between means are indicated as * p <
591 0.05. Values in bold indicate that is the highest value for a given variable (in a row).

592

	Peña Hueca		Villacañas	
	Me	**M ± SD**	**Me**	**M ± SD**
Na	6.04	30.1 ± 41.9	6.39	6.45 ± 2.59
K	1.66	1.94 ± 0.493	1.72	1.68 ± 0.316
Mg	1.16	0.954 ± 0.439	1.02	0.979 ± 0.075
Cu*	**0.940**	**0.784 ± 0.322**	0.250	0.255 ± 0.088
Zn	0.477	0.421 ± 0.212	0.462	0.383 ± 0.239
Mn	0.170	0.173 ± 0.123	0.138	0.154 ± 0.047
Ca	0.071	0.072 ± 0.016	0.050	0.046 ± 0.022
Rb	0.071	0.073 ± 0.014	0.079	0.078 ± 0.006
Sr	0.055	0.055 ± 0.012	0.051	0.069 ± 0.055
Fe	0.012	0.012 ± 0.002	0.014	0.011 ± 0.007
Co	0.017	0.032 ± 0.034	0.023	0.026 ± 0.004
Ni	0.036	0.038 ± 0.011	0.055	0.055 ± 0.008
Ba	0.007	0.008 ± 0.005	0.013	0.011 ± 0.005
Pb	0.010	0.010 ± 0.003	0.003	0.006 ± 0.007

593

594

595 **Table 8:** Bioconcetration factor (BCF). Data expressed as mean ± standard deviation. n=3. Statistically significant
596 differences between means are indicated as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different lower-case letters
597 indicate statistically significant differences between means of the same variable. Values in bold indicate the
598 highest values for a given variable (in a row).

	Spring	**Summer**	**Autumn**	**Winter**
			Peña Hueca	
K	130 ± 68.4	76.3 ± 50.8	168 ± 102	254 ± 104
Na	25.0 ± 2.82	45.5 ± 64.5	32.0 ± 8.1	134 ± 109
Mg	9.79 ± 1.04	14.8 ± 20.8	10.1 ± 1.6	24.1 ± 4.2
Ca	20.5 ± 1.2	15.6 ± 2.3	16.1 ± 1.0	23.7 ± 9.7
			Villacañas	
K ***	43.0 ± 15.2[a]	21.6 ± 10.8[a]	26.1 ± 7.3[a]	**212 ± 62[b]**
Na ***	11.1 ± 2.14[a]	10.2 ± 3.4[a]	9.63 ± 1.24[a]	**191 ± 65[b]**
Mg ***	4.50 ± 1.32[a]	2.74 ± 1.06[a]	2.18 ± 0.58[a]	**42.5 ± 13.6[b]**
Ca	15.1 ± 3.46	12.8 ± 7.6	10.1 ± 4.3	15.2 ± 4.5

599

Figure captions

Fig.1: Location of the sampling sites of this study. (a) Map of the Iberian Peninsula and location of Toledo province. (b). Location of Laguna Larga de Villacañas and Peña Hueca in the Toledo province. In light gray villages, in dark grey diverse lagoons in the area. (c) Sampling area of Laguna Larga de Villacañas. The lightest gray shows the area occupied by *S. carinata* community. Points show the approximate location of the three sampled sites. (d) Sampling area of Peña Hueca. The lightest gray shows the area occupied by *S. carinata* community. Points show the approximate location of the three sampled sites

Fig. 2: Biplot of the variables studied and the two-principal component (PC1, PC2). The black lines represent the variables studied in the plants. Those with a bold and underlined label contribute the most to PC1 and PC2 components. Gray diamonds and gray labels with an asterisk represent environmental and edaphic variables.

Fig. 3: Representative XRD diffractograms of the succulent stem (a) and woody stem (b) of *Sarcocornia carinata*. The numbers close to the peaks refer to the crystallographic planes (hkl).

Fig. 4: Representative SEM images of the succulent stems of *Sarcocornia carinata*. (a). Cross section of a succulent stem. (b). Details of the central cylinder in a cross section. ep: single cell layer epidermis; cc: central cylinder; p: parenchyma cells of the central cylinder; ph: phloem; pp: palisade parenchyma; tr: tracheoid idioblasts distributed throughout the palisade parenchyma and the water storage parenchyma; ve: vessel; wsp: water storage parenchyma; x: xylem. Bars: (a) 500 µm; (b) 100 µm.

Fig. 5: Representative SEM images of the succulent stems of *Sarcocornia carinata*. (a) and (b). Cross sections of the succulent stems of a sample from the winter (a) and another from the summer (b). There are bright white precipitates in the water storage parenchyma and a central cylinder in both images; (c). A detail of the precipitates is signalled in photograph b by a white arrow; (d). Detail of the water storage parenchyma collapsed by salty precipitates; (e). Detail of a cross section of a central cylinder with several vessels collapsed by salty precipitates; (f). Detail of a longitudinal section of a central cylinder with vessels collapsed by salty precipitates; (g). Detail of the different appearance of the salty precipitates of a cell in the water storage parenchyma; Lowercase letters a-e indicate the places where EDX analyses of Fig. 5 have been carried out. Bars: (a) 500 µm; (b) 1mm; (c) 50 µm; (d) 200 µm; (e) 30 µm; (f) 30 µm; (g) 50 µm.

Fig. 6: EDX analysis of samples shown in Fig.4. Each spectrum corresponds to the same lowercase letter as the images in Fig. 4.

Fig. 7: Representative SEM images of different crystallizations found in the tissues of *Sarcocornia carinata*. (a). Longitudinal section of the cortical tissue of a woody stem. A large number of crystals can be seen in most of the cells; (b). A cell in the water storage parenchyma of a succulent stem containing numerous crystallizations; (c). Detail of the polyhedral crystals shown in (a). They are composed of O and Ca (Fig.7a); (d). Detail of polyhedral crystals shown in (b). They are composed of O and Mg (Fig.7b); (e). A druse composed of multiple prismatic Ca crystals; (f). Multiple crystals in a parenchymatic cell from a succulent stem. The big grey amorphous material is composed of O and Mg (Fig.7c) while the long, fine and brighter crystals are composed of O, Ca and S (Fig.7d); Lowercase letters a-d indicate the places where EDX analyses of Fig. 7 have been carried out. Bars: (a)100 µm; (b) 50 µm; (c) 20 µm; (d) 10 µm; (e) 50 µm; (f) 10 µm;

Fig. 8: EDX analysis of samples shown in Fig.6. Each spectrum corresponds to the same lowercase letter as the images in Fig. 6.