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IN SITU STUDIES ON SEA-SALT UPTAKE BY EPIPHYTIC LICHENS

In situ-Untersuchungen über die Meersalzaufnahme von epiphytischen Flechten

by

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Schlagwörter: Biomonitoring, zelluläre Lokalisierung, Flechten, *Ramalina canariensis*, Meersalzspuren, stufenweise Elution.

Summary: The deposition of saline particles on lichens and the cellular location of cations were followed on field samples of *Ramalina canariensis*, growing on the main stem of pines at ca. 800 m from the coast in southern Portugal. Some lichen thalli were rinsed with deionised water. Extracellular and intracellular concentrations of Na^+, K^+ and Mg^{2+} were determined after several periods of exposure to natural sea spray, up to one month. The same determinations were made on unrinsed control samples. Extracellular concentrations of Cl^- were measured in both samples as well. Rinsed lichens exhibited lower extracellular Cl^- and Na^+ values up to the 15^{th} day of the experiment. From this date onwards, extracellular concentrations of Cl^- and Na^+ were not significantly different, showing a rapid recovery to natural levels in washed samples.

Zusammenfassung: Die Deposition von Salzpartikeln auf Flechten und die zelluläre Lokalisierung der Kationen wurde an Freilandproben von *Ramalina canariensis*, die auf dem Hauptstamm von Föhren in 800 Metern Entfernung von der Küste des südlichen Portugal wuchsen, untersucht. Einige Flechtenthalli wurden mit deionisiertem Wasser gespült. Die extra- und intrazellulären Konzentrationen von Na^+, K^+ und

Mg^{2+} wurden nach unterschiedlich langen Expositionen, die bis zu einem Monat betragen konnten, untersucht. Dieselben Untersuchungen wurden an ungespülten Kontrollproben durchgeführt. Die extracellulären Konzentrationen von Cl^- wurden in den ungespülten und gespülten Proben bestimmt. Die gespülten Proben wiesen bis zum 15. Tag des Experiments geringere extracelluläre Cl^- und K^+-Werte auf. Nach diesem Zeitraum waren die extracellulären Konzentrationen von Cl^- und K^+ nicht signifikant unterschiedlich, was auf eine rasche Rückkehr der gewaschenen Proben zu den natürlichen Werten hinweist.

Introduction

Compared with other air pollutants, such as heavy metals, radionuclides, organic compounds, etc. (Brown & Di Meo 1972; Larson et al. 1986; Takala et al. 1990; Figueira et al. 1995), saline elements have received little attention in lichen biomonitoring studies. Although airborne salinity is not viewed as a typical air pollutant, it has an enormous impact, especially on coastal areas, as a factor of degradation or corrosion of materials, with negative effects also on soil and aquifer contamination. Sea-salt biomonitoring through lichens may prove to be valuable in assessing risk factors associated with deposition of airborne salts.

In order to use lichens as saltfall biomonitors, it is important to gather field information on the response time of lichens to changes in atmospheric concentrations of saline elements, and on the influence of climatic factors in uptake processes. Additional information is also required on the mechanisms involved in the uptake of saline elements, and on the possible physiological effects caused by exposure to high concentrations. The knowledge of the cellular location of such elements is also essential to understand these processes. The present study was designed to follow the concentration of saline elements in field samples of a lichen, *Ramalina canariensis* Steiner, within short periods of time. The main aim was to determine whether biomonitoring results concerning salt deposition indicate long-term deposition phenomena or just reflect short- term events. Another goal was to study the variation in salt concentrations within surface and intercellular, wall-bound, and intracellular fractions of the lichen.

Materials and Methods

Field procedures: The study was carried out between February 28 and April 1, 1996. Several pine trees *(Pinus pinaster)* with *Ramalina canariensis* were selected from a site at about 800 m from the coast. Lichen thalli growing on the west and north-west side of the main stems, facing the shoreline, and between 2 and 4 m from the ground, were rinsed with about 500 ml of bidistillated and deionised water. Lichen samples were collected immediately after rinsing, and following several periods of exposure: 1, 3, 7, 15, 22 and 33 days. Samples of

unrinsed thalli were also collected at the same dates, and considered as control material. All samples were dried at room temperature and stored in a cold chamber (4° C) for one month (at maximum) before laboratory processing.

Laboratory procedures: The method for the determination of cellular fractions of cations followed the sequential elution technique by BROWN and WELLS (1988), with some modifications. Prior to elution, complete thalli (about 50 mg) were stored for 24 hours in a high humidity chamber. The first fraction to be eluted was the total extracellular one, which includes elements located on the surface, in the intercellular spaces, and bound to the cell. This fraction was determined by shaking samples in two plastic flasks with 10 and 5 ml of $NiCl_2$ (20 mM) for 40 and 30 minutes, respectively. Elements bound to the cell walls were determined by the same procedure; yet, prior to nickel chloride shaking, samples were washed thoroughly with deionised water (1 l) to remove unbound elements from the surface. The fraction of surface and intercellular elements was determined by the difference between total extracellular and bound elements. Afterwards, samples were dried at 80° C for 16 h before measuring dry weight (d.w.). The intracellular fraction was eluted by shaking samples for 1 h 30 min in 1M HNO_3. Element concentrations were measured by atomic absorption spectrophotometry. Surface chloride contents was determined by shaking ca. 50 mg of lichen samples twice in plastic flasks with 10 ml deionised water. The dry weight was determined after drying at 80° C for 16 h. Chloride was measured by mercurimetric titration (SCHALES & SCHALES 1941). The results were expressed in µmol/g d.w., and are based on ten independent samples.

Results and discussion

The rinsing of lichen thalli with deionised water lowers the total extracellular Cl^- and Na^+ by five and three times, respectively (Fig. 1). The extracellular concentration of Mg^{2+} was also decreased to a lesser extent (about two times) but K^+ remained at control level. The values for the rinsed samples were constant up to the 7^{th} day after rinsing. Between the 7^{th} and the 15^{th} day, an increase was observed in the concentration of the rinsed samples to near the control values of Cl^-, Na^+ and Mg^{2+}.

After the 15^{th} day and up to the end of the sampling program, the extracellular values of the rinsed samples were at the control levels. As observed before, the control values showed considerable oscillation, especially for Cl^- and Na^+, sometimes duplicating the extracellular concentration for a short period (one week for Cl^- and three weeks for Na^+): this means that the concentration of such elements may change very rapidly.

The cellular location of the elements was determined, and showed very

different patterns, depending on the ion considered (Fig. 2). The fluctuation of element levels was also different, and it depended on the cation and its cellular location. In general, surface and intercellular fraction was the most variable for all elements, both in control and rinsed samples. Most Na^+ is present in the surface and intercellular fraction, with large variations in both sample sets. Initial rinsing produced a substantial decrease in this fraction, but a fast recovery to the control levels occurred between the 7^{th} and the 15^{th} day of exposure. Also, a decrease of extracellular elements bound to the cell wall was initially observed, yet to a lesser extent, showing a much more stable behaviour of this fraction.

In contrast with Na^+, most Mg^{2+} detected in the lichen thalli were bound to the exchange sites of cell walls at a much higher concentration than in the surface fraction. These results show the higher affinity of magnesium ions to the negative charged groups present on the cell wall, as also reported for bryophytes (BROWN & WELLS 1988). The different affinity of the elements to the wall anionic groups can bias the correspondence between biomonitoring values and atmospheric concentrations (BROWN & BROWN 1991). The surface and intercellular Mg^{2+} fractions exhibited an increase after the 7^{th} day of the experiment, as in the case of Na^+. However, the concentration of intracellular Mg^{2+} was very similar in both rinsed and control samples.

As for K^+, the most important fraction was found to be the intracellular one, a fact which was also observed in other lichen species (BRANQUINHO & BROWN 1994). No significant differences were observed between rinsed and control samples as to the amount of K^+ in the intracellular and wall-bound fractions. About 80% of total K^+ was measured within the cell for both sets of samples, in almost every period of exposure. The fact that the inner fraction of K^+ is near the same value in either group may indicate that no important damage was induced to the cell membrane, a fact that could cause some loss of K^+ from the cell (BUCK & BROWN 1979; BROWN & BROWN 1991). The surface fraction, as observed for the other elements, showed lower values in the onset of the study, followed by a rapid recovery after the 7^{th} day.

Conclusions

The extracellular concentrations of saline elements showed frequent variations within short periods of time, especially for elements in the surface and intercellular fraction. The equilibrium level of this fraction was reached by rinsed lichens in 15 days, which means a fast recovery of the extracellular concentrations under field conditions. The fraction of the elements bound to the cell walls shows a lesser variation in a short-time scale. For this fraction, the ionic properties of the elements may bias the direct indication of atmospheric deposition by lichens.

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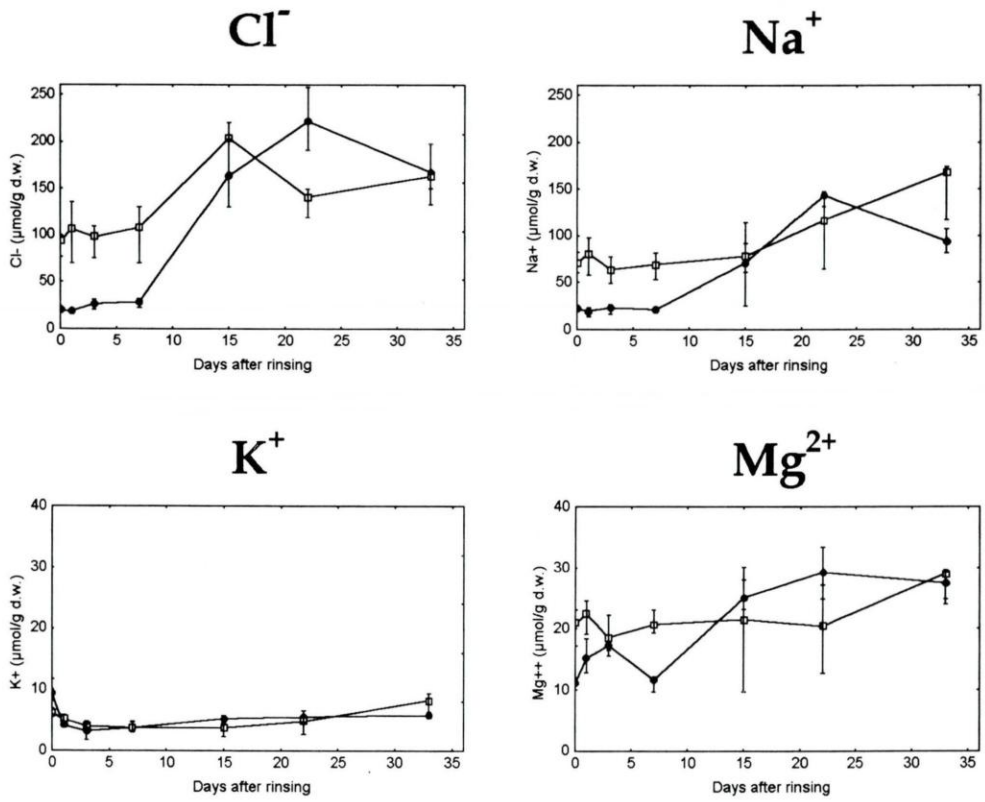

Fig. 1: Total extracellular concentrations in rinsed and control samples, measured at start and after 1, 3, 7, 15, 22 and 33 days after rinsing. (●) - rinsed; (□) - control. Bars represent the interquartile range.

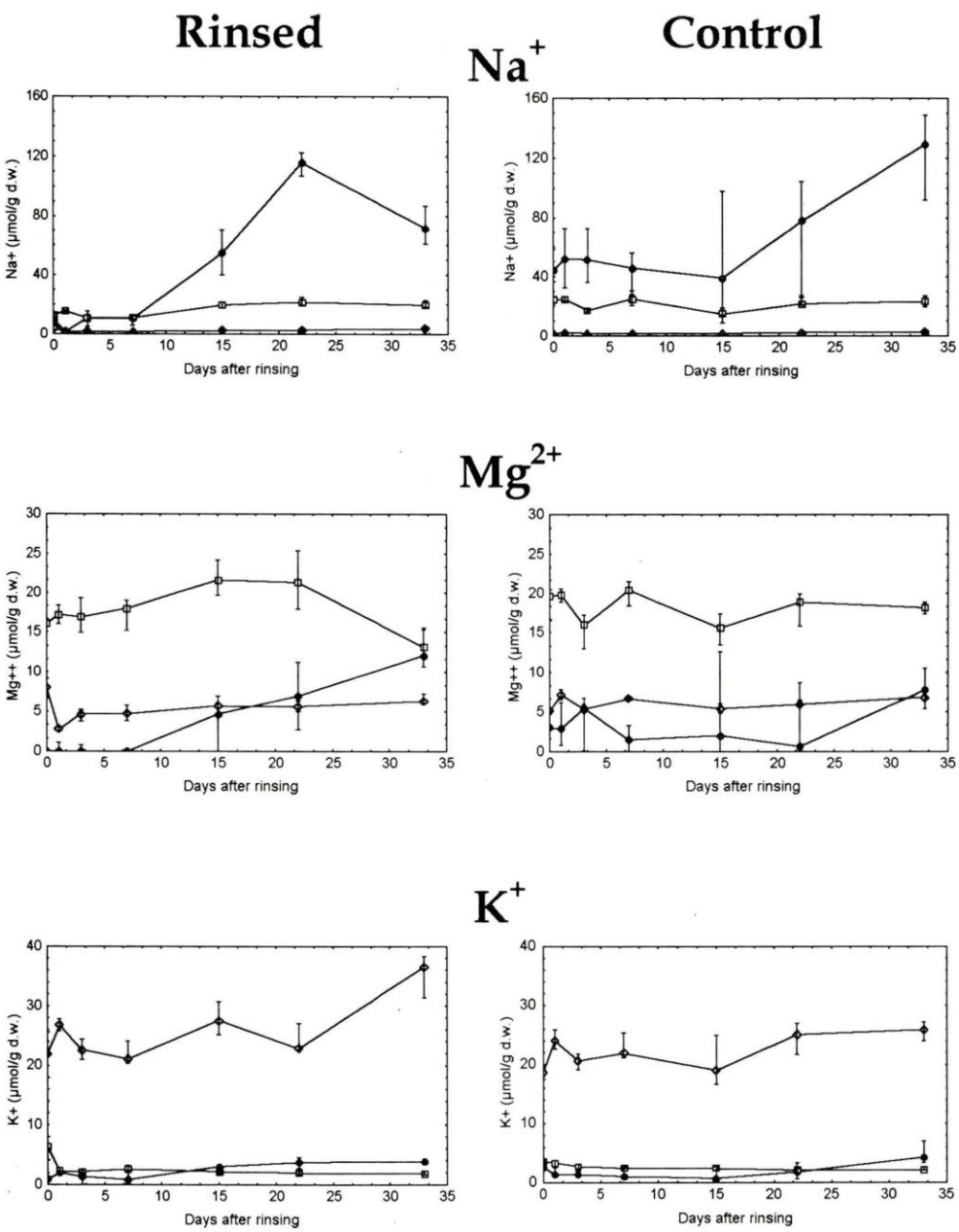

Fig. 2: Element concentrations in the different cellular fractions of rinsed and control samples over the sampling time. (●) - surface and intercellular; (□) - wall-bound; (◇) - intracellular. Bars represent the interquartile range.