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Development and calibration of epiphytic lichens as saltfall biomonitors—dry-deposition modelling

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"Capsule": *The concentration of saline elements measured in lichens was calibrated against dry deposition.*

Abstract

Lichen transplants (*Ramalina calicaris* and *Usnea* spp.) were investigated as biomonitors of the atmospheric deposition of marine salt, and a calibration model was set up to predict the dry deposition of saline elements from the concentration of salt tracers in lichens. The study was performed in the Portuguese Atlantic coast, where a monthly transplantation program was run in two stations that show clear differences in terms of precipitation regimes. At both stations, dry deposition and precipitation records were kept for the whole duration of the program. General trends in results have indicated that rainwater may wash saline elements out of lichens, though such an effect does not appear to be linear. A multiple-regression approach was taken to look for a calibration between dry-deposition fluxes and lichen concentrations through a stepwise technique. The calibration model for data obtained in both stations features two break points that define precipitation ranges for low, moderate and heavy rain conditions. The results show that lichens can really be used to indicate the dry deposition of sea salt. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Lichen transplants; Biomonitoring; Calibration; Tropospheric salinity; Atmospheric deposition; Precipitation

1. Introduction

Lichen biomonitoring is an interesting tool for environmental assessment, with plenty of applications in the evaluation of most notorious contaminants, such as heavy metals, trace elements, radionuclides and nitrogen/sulphur oxides. Some of these contaminants result from anthropogenic activities, which the majority of publications in the field are devoted to. Compared to this, little attention has been paid to the study of sea-salt deposition inland—a natural vector of contamination—despite its hazardous consequences on terrestrial areas, ranging from materials failure to soil and water impoverishment and desertification at large (Pacheco et al., 1995). With the application of biomonitors, in particular lichens and mosses, it as been possible to implement large monitoring programs, at regional (Figueira et al., 1999a) or continental (Rühling and Steinnes, 1998) levels. The costs associated with these campaigns are much lower than the implementation of sampling networks using physico-chemical devices.

Biomonitoring studies are however subjected to the availability of the biomonitor in the field. In cases where the biomonitor species cannot be found in sufficient amounts at the sampling locations, transplants can be used to obtain relative values of the atmospheric deposition. In this case, the organisms are normally collected from a remote site, not subjected to the contaminant that is intended to monitor, and exposed to that factor in the new location. Transplants were used to monitor the atmospheric deposition of particles caused by mining activity (Pereira et al., 1995), contamination by radionuclides (Jeran et al., 1995), or other pollutants (Pilegaard, 1979; Garty et al., 1982; Loppi et al., 1998).

The location of mineral elements on lichens is extracellular or intracellular. The extracellular elements can be located on the surface and intercellular spaces, or bound to cell wall exchange sites (Brown and Buck, 1985). Surface elements are simply deposited, without

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any ionic binding to lichen structures. This fraction can represent the bulk of the elements present in extracellular compartments of the lichen thalli, as observed for Na in lichens collected on a coastal area (Figueira et al., 1999b). Similarly, most airborne chlorides should be deposited on the surface as well. The mobilisation of these elements, that may be in particulate form, can be greatly dependent on climatic conditions, therefore all these factors should be considered when the use of lichens as saltfall biomonitors is attempted.

The accumulation of airborne elements by lichens' cell-wall—particularly, cations—is known to be dependent on the cation-exchange capacity of the cell wall, in which negatively-charged binding sites establish an interaction with cations. This is one of the factors that enables them to accumulate elements in quantities several times higher than their nutritional requirements (Nash III, 1996). Anionic uptake is less understood, though it is generally accepted that the anionic-exchange capacity of lichens is much lower (Nash III, 1996). Nonetheless, dissimilar cations show different affinities to the exchange sites. In general terms, such an affinity can be ordered in the sequence found by Nieboer and Richardson (1980), based on competitive experiments: monovalent Class A $<$ divalent Class A $<$ Borderline divalent $<$ divalent Class B. According to this order, Na and Mg, which are among the most abundant elements in sea water, are classified as monovalent class A and divalent class A, respectively. This means that the capacity of retention of such elements is limited if other cations are present in plentiful supply in the environment, as they are likely to be displaced through competition for binding sites.

The intracellular elements are taken up through the cell membrane, by passive or active transporting mechanisms, involving systems with different levels of affinity. The intracellular concentration is also subjected to electrochemical balance. It is, therefore, highly controlled by physiological processes, which means that cytoplasm concentration of elements hardly corresponds to external variations of element availability from atmospheric deposition (Brown and Brown, 1991). In studies where total contents of elements are determined, the lack of discrimination between levels in different cellular compartments can be a limitation, because intracellular values may bias the interpretation of biomonitoring values.

The aim of this study is to assess and improve the use of lichens as biomonitors of the atmospheric deposition of sea salt, by means of a calibration between lichen accumulation and dry deposition of saline elements. The work was developed using lichen transplants collected from a non-coastal site and exposed during 1-month periods at two meteorological stations near the coast. The importance of precipitation was assessed by the selection of two transplant sites, with large differences in precipitation regimes. The importance of this factor in the uptake and release of elements by lichens was clearly identified and included in the calibration model, following a stepwise approach.

2. Material and methods

Two atmospheric stations with strong differences between their annual precipitation regimes were selected on the Portuguese west coast (Fig. 1). The first one is located in Monte Velho (MV), south of Lisbon, and features an annual precipitation below 600 mm; its distance from the shore is about 1 km. The second station is located in Meadela (ME), north of Oporto, some 4 km from the coast, and it shows rainfall averages above 1400 mm per year. Samples of the lichens *Ramalina calicaris* and *Usnea* spp. were collected at a remote, clean site in southern Portugal, approximately 18 km from the coast, and transplanted into both stations on a monthly-exposure basis. The first species was sampled on olive trees (*Olea europaea*), and the second one on cork oaks (*Quercus suber*). Transplants were exposed by attaching the original phorophyte branches to a wood stand installed in either station, using a nylon thread. After 1 month, the lichen thalli were removed to the laboratory, cleared of wood and other alien materials, and then processed for the determination of element concentrations.

Surface Cl^- was determined by shaking ca. 50 mg of lichen samples in plastic flasks twice, with 10 ml of deionised water each time. The leachates were assessed by mercurimetric titration (Schales and Schales, 1941). The weight of each sample was determined after drying at 80 C for 16 h.

The determination of extracellular, cationic (Na^+ and Mg^{2+}) loads generally followed the sequential-elution technique by Brown and Wells (1988), with some modifications though. Prior to elution, each sample of approximately 50 mg of intact thalli had been stored for 24 h at 100% relative humidity. The total extracellular fraction, that includes all ions over the surface, within intercellular spaces and exchangeably bound to cell walls, was obtained by shaking samples in plastic flasks with 10 and 5 ml of $NiCl_2$ (20 mM) for 40 min and 30 min, respectively. Samples were dried at 80 C for 16 h before weighing. $[Na^+]$ and $[Mg^{2+}]$ were measured by atomic absorption spectrometry.

All results are given in micromole per gram of dry weight (μmol g^{-1} dw) of lichen, and they are based on 10 independent samples for each transplantation site. In addition to biological data, dry deposition and precipitation data were supplied for each station by the Portuguese Institute of Meteorology and an official, environmental agency (DRARN-Alentejo), for the whole extent of the exposure program.

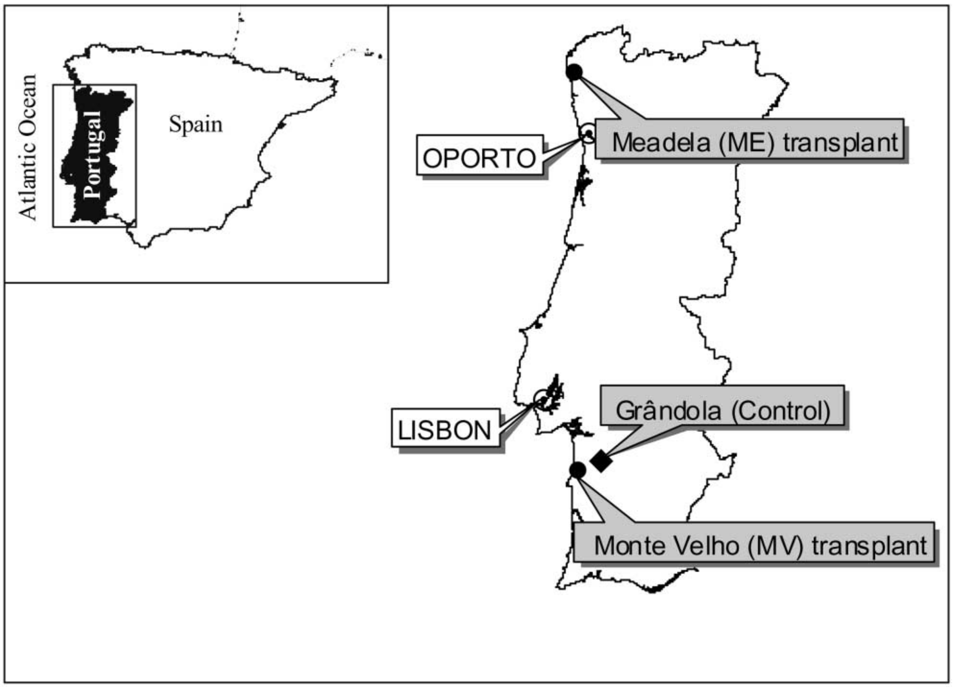

Fig. 1. Map of mainland Portugal, showing the approximate location of sampling (control) and exposure (transplant) sites.

3. Results and discussion

In previous studies, the factorial analysis of element concentration in indigenous lichens, sampled for mapping purposes, showed that chloride, sodium and magnesium are important elements in the definition of a salinity index, and can be interpreted as sea-salt tracers (Figueira et al., 1999c). Using information about all three elements may improve the calibration model, because the biological interactions between the elements in the different cellular fractions, are reflected in the results obtained. Moreover, the calibration between dry deposition and biomonitoring should include in the model other factors that interact with the uptake of atmospheric elements by lichens, in which precipitation is one of the most important.

The biological values correspond to differences between pre- and post-exposure concentrations, therefore any negative value indicates loss of an element. The reason for this loss can be related to the washout effect of precipitation, giving an indication of the importance of such a factor in the process of accumulation of airborne elements by lichens. Because different washout effects should result from different rain intensities, values of precipitation were weighted by the time length of rain events (in days), on a monthly basis, in order to produce a rain intensity measurement, which was called Reduced Average Precipitation (RAP, in mm/day), defined as described in Eq. (1).

$$\mathrm{RAP} = \frac{P}{\mathrm{DP}} \tag{1}$$

where P is the amount of precipitation measured in a month, and DP represents the number of days in a month in which were observed rain events.

The transplant program first started at MV station, in September 1993, where it was run until the end of 1997: these results are presented in Fig. 2. It can be observed that *R. calicaris* and *Usnea* spp. clearly show some different ability to accumulate saline elements, but the temporal variation is consistent between the two species, as proved by the significant correlation coefficients ($r = 0.61$ for Cl^-, $r = 0.67$ for Na^+ and $r = 0.55$ for Mg^{2+}). Such variation in time also seems to match the change in rainfall intensity observed throughout the study. Generally speaking, periods with higher precipitation rates correspond to lower extracellular concentrations of saline elements on lichens, and the contrary seems to apply for drier months, as can be observed by comparison between RAP values and biological data (Fig. 2). Other studies conducted in the area, using indigenous *Ramalina canariensis*, have shown seasonal variations of the Cl^- and Na^+ contents, in good agreement with RAP values, and without delay effect between the elements and precipitation (Figueira et al., 1999b). Also on *Ramalina menziesii* it was observed that leachable ion concentration were significantly higher in summer than in winter (Boonpragob et al., 1989).

The precipitation events may still promote the accumulation of saline elements by lichens, for low amounts of rain that fail to leach the lichen thalli thoroughly. In such cases, elements in rainwater may be accumulated by lichens, as well as elements from particles dissolved

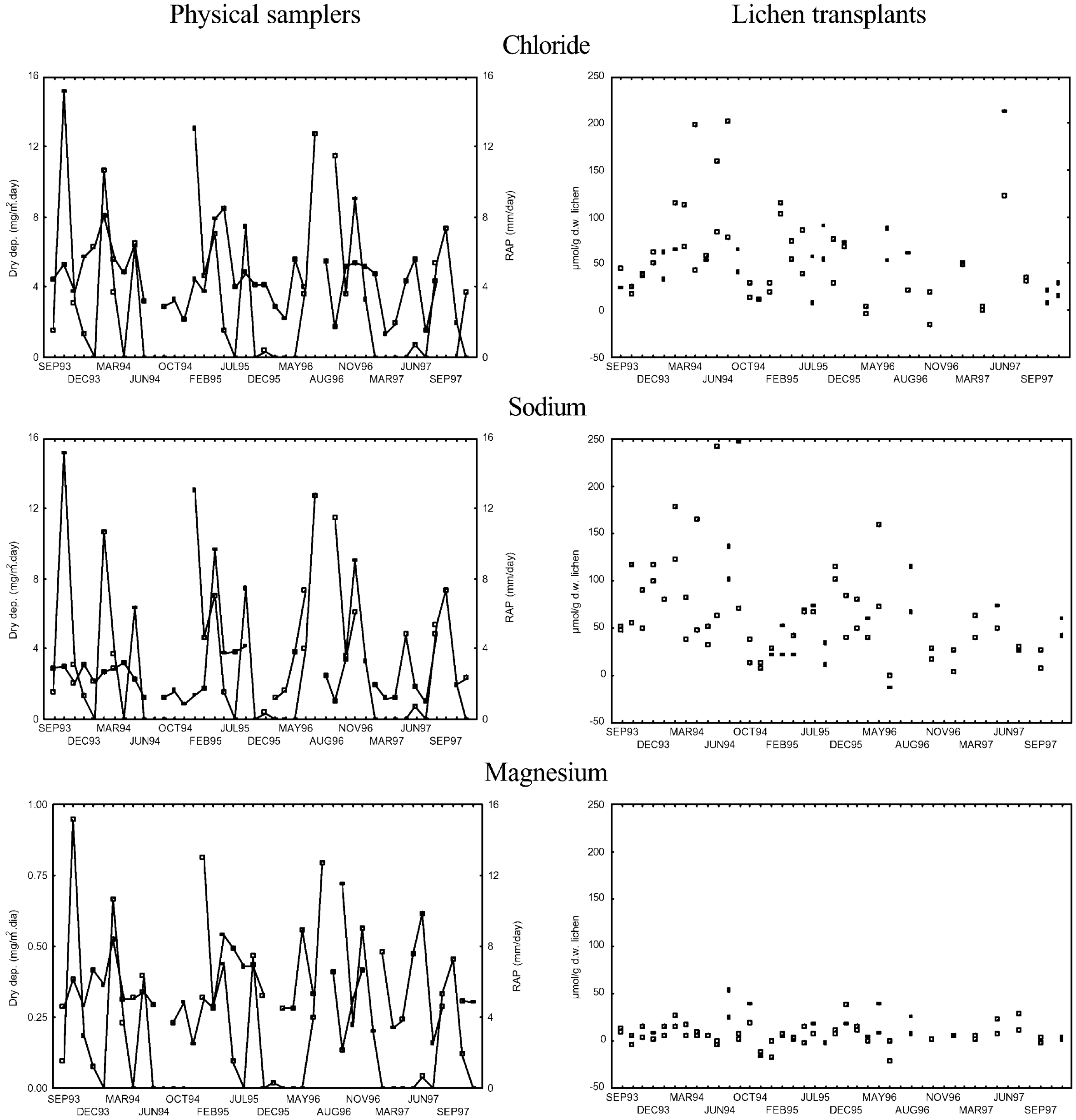

Fig. 2. Time profiles of physical (left) and biological (right) data obtained at MV station. In physical-data patterns, solid symbols are for dry deposition and open ones are for RAP. In biological-data patterns, solid symbols are for *Usnea* spp. and open ones are for *Ramalina calicaris*.

by rain, that have been trapped on the surface and into intercellular spaces. All of them may become available for the establishment of van der Waals interactions with the exchange sites over the cell wall, or even for an uptake by the cell itself.

However, there is no definite linear relationship between the amount of rain and the concentration of elements in lichens, as demonstrated by the low and non-significant correlation coefficients (Table 1). This can mean that such a relationship features some discontinuity, resulting from the action of different intensities of rain on extracellular elements deposited on lichens. For high precipitation rates, the leaching and washout effects of rainwater should act more like removing elements from the lichen surface, and to some extent, from exchangeable sites, at least for elements with lower ionic affinities with such sites in the cell wall. About 80% of the sodium in the extracellular fraction is deposited on the surface or trapped into intercellular spaces (Figueira et al., 1999c), and it can be easily leached away from lichens by distilled water (Figueira et al., 1998). The washout of nutrients from lichen thalli by distilled water has already been observed in former experiments (Kytöviita and Crittenden, 1994). The effect of long periods of rain could be similar to the effect of distilled water, at least when the rain events last for long enough to decrease the element concentrations in rainwater (Beverland and Crowther, 1992).

Table 1
Correlation coefficients between RAP and the concentration of each element on lichens ($n=32$)

	Usnea spp.			*Ramalina calicaris*		
	Cl^-	Na^+	Mg^{2+}	Cl^-	Na^+	Mg^{2+}
RAP	−0.35	−0.31	−0.51*	−0.28	−0.21	−0.21

* r significant at $P<0.05$.

These considerations indicate that precipitation is an important factor to be accounted for in putting together a calibration model that estimates atmospheric deposition of saline elements from lichen data. The model to be built will allow the estimation of dry deposition of saline elements (chloride, sodium and magnesium), and will rely on the following design:

- chloride, sodium and magnesium, used together as salt tracers, describes better the uptake of saline elements by lichens, and should be used jointly as explanatory variables;
- precipitation may raise/drop the accumulation of saline elements by lichens, therefore can be used as explanatory variable.

The interaction of precipitation on lichen uptake may display, however, non-linear features. Still, the relationship can be linearly modelled in the ranges of precipitation where the effect is only of one type. The parametric definition of the trigger value that switches the effect of rain from an increase to a decrease in element uptake by lichens is quite difficult, and, arguably, it can only be done for specific conditions, as other ecological and environmental factors may also influence the uptake process—for instance, air humidity, wind regime and lichen morphology, to mention only a few. Therefore, an iterative approach was taken, which includes the biological concentrations of Cl^-, Na^+ and Mg^{2+}, and RAP. The break point of RAP, found for the model by an iterative approach in order to obtain the best variance explanation, is 5.5 mm day^{-1}, as given in Eq. (2):

$$[X]_{\mathrm{atm}}=\begin{cases} a_0+a_1[Cl^-]_{\mathrm{bio}}+a_2\left[Na^+\right]_{\mathrm{bio}} \\ \quad +a_3\left[Mg^{2+}\right]_{\mathrm{bio}}+a_4\mathrm{RAP}; & \mathrm{RAP}<5.5 \\ b_0+b_1[Cl^-]_{\mathrm{bio}}+b_2\left[Na^+\right]_{\mathrm{bio}} \\ \quad +b_3\left[Mg^{2+}\right]_{\mathrm{bio}}+b_4\mathrm{RAP}; & \mathrm{RAP}\geqslant 5.5 \end{cases} \tag{2}$$

where $[X]_{\mathrm{atm}}$ stands for the dry deposition of salt tracer X, estimated in mg m^{-2} day^{-1} and $[X]_{\mathrm{bio}}$ stands for the concentration of element X in the saltfall biomonitor, measured in μmol g^{-1} dw.

The corresponding coefficients are presented in Table 2. The model seems to yield acceptable results, in particular for *R. calicaris*. The plots of predicted vs. observed values of dry deposition for all three elements can be found in Fig. 3, from where it can be concluded that no systematic bias is evident in the results of the model.

The study was also run at the ME meteorological station, where it was possible to test the lichens as saltfall biomonitors in very different precipitation regimes. The transplantation program started in October 1996 and lasted until June 1998; its modus operandi was the same as before (MV station). Fig. 4 shows the corresponding values for physical and biological data. Now, *Usnea* spp. seems to have a superior ability to accumulate saline elements than *R. calicaris*. This may be a consequence of a higher availability of elements in ionic form, due to the frequent occurrence of rain events that make elements readily available for uptake by cell walls. Laboratory observations of cation uptake by these species, when incubated into artificial seawater, have already pointed to a higher cation-exchange capacity of *Usnea* spp. as regards Na^+ and Mg^{2+} (Figueira et al., 1994). For Cu, laboratory studies have also indicated a higher cation exchange capacity of the *Usnea* species when compared to *Ramalina fastigiata* (Branquinho et al., 1997), and field studies using transplants around a copper mine have confirmed these results (Branquinho, 1997).

It was also found that the difference between original and terminal concentrations was often negative, confirming the mobilising action of rain on the extracellular elements. The type of calibration model tested for MV was also implemented for ME, and the analysis of results showed that a different break point had to be established, in order to adjust the model to the higher RAP values observed in this station. The value of the break point that came out is 15 mm day^{-1}, and the coefficients of the model described in Eq. (3) are displayed in Table 3.

$$[X]_{\mathrm{atm}}=\begin{cases} a_0+a_1[Cl^-]_{\mathrm{bio}}+a_2\left[Na^+\right]_{\mathrm{bio}} \\ \quad +a_3\left[Mg^{2+}\right]_{\mathrm{bio}}+a_4\mathrm{RAP}; & \mathrm{RAP}<15 \\ b_0+b_1[Cl^-]_{\mathrm{bio}}+b_2\left[Na^+\right]_{\mathrm{bio}} \\ \quad +b_3\left[Mg^{2+}\right]_{\mathrm{bio}}+b_4\mathrm{RAP}; & \mathrm{RAP}\geqslant 15 \end{cases} \tag{3}$$

In the above equation, $[X]_{\mathrm{atm}}$ stands for the dry deposition of salt tracer X, estimated in mg m^{-2} day^{-1} and $[X]_{\mathrm{bio}}$ stands for the concentration of element X in the saltfall biomonitor, measured in μmol g^{-1} dw.

The model seems to fit well with the experimental values, producing good estimates as confirmed by the correlation coefficient, generally above 0.9, and this result could be obtained for both lichen species. Plots of

Table 2
Regression coefficients (a_i, b_i) and correlation coefficients (r) of the calibration model for lichen species in MV station

	a_0	a_1	a_2	a_3	a_4	b_0	b_1	b_2	b_3	b_4	r
Ramalina calicaris											
Chloride	1.719	0.007	0.019	0.057	0.443	6.875	−0.007	0.019	0.103	−0.153	0.74
Sodium	1.669	0.001	0.004	0.038	0.082	2.456	0.022	0.001	−0.022	−0.075	0.46
Magnesium	0.214	0.000	0.001	0.006	0.014	0.389	0.001	0.000	0.007	−0.003	0.72
Usnea spp.											
Chloride	3.158	0.007	0.000	0.008	0.255	7.179	−0.008	0.014	0.063	−0.218	0.63
Sodium	1.669	0.001	0.004	0.038	0.082	2.456	0.022	0.001	−0.022	−0.075	0.40
Magnesium	0.214	0.000	0.001	0.006	0.014	0.389	0.001	0.000	0.007	−0.003	0.54

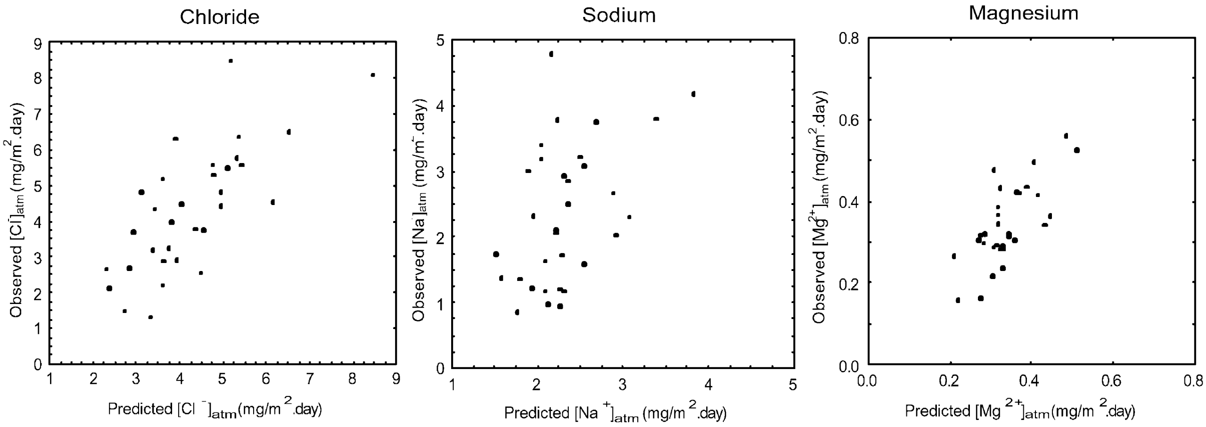

Fig. 3. Results of the calibration model of *Ramalina calicaris* for Cl^-, Na^+ and Mg^{2+} at the MV station.

predicted vs. observed values for *R. calicaris* also confirm that no systematic bias underlies the calibration results (Fig. 5).

The manifest difference in rainfall between stations allows the study of two different situations that more or less make the extremes of what can be found in Portugal, in terms of precipitation regimes. This enables the calculation of a global model that covers the whole range of precipitation conditions, by joining the two data sets and using a calibration model with break points for both low and high precipitation rates, calculated for each station. A general-purpose model was calculated using the break points previously found, as displayed in Eq. (4)

$$[X]_{\mathrm{atm}} = \begin{cases} a_0 + a_1[Cl^-]_{\mathrm{bio}} + a_2[Na^+]_{\mathrm{bio}} \\ \quad + a_3[Mg^{2+}]_{\mathrm{bio}} + a_4\mathrm{RAP}; & \mathrm{RAP} < 5.5 \\ b_0 + b_1[Cl^-]_{\mathrm{bio}} + b_2[Na^+]_{\mathrm{bio}} \\ \quad + b_3[Mg^{2+}]_{\mathrm{bio}} + b_4\mathrm{RAP}; & 5.5 \leqslant \mathrm{RAP} < 15 \\ c_0 + c_1[Cl^-]_{\mathrm{bio}} + c_2[Na^+]_{\mathrm{bio}} \\ \quad + c_3[Mg^{2+}]_{\mathrm{bio}} + c_4\mathrm{RAP}; & \mathrm{RAP} \geqslant 15 \end{cases} \tag{4}$$

where $[X]_{\mathrm{atm}}$ stands for the dry deposition of salt tracer X, estimated in mg m^{-2} day^{-1} and $[X]_{\mathrm{bio}}$ stands for the concentration of element X in the saltfall biomonitor, measured in μmol g^{-1} dw.

The coefficients are listed in Table 4. This model was set up for *R. calicaris* only, owing to the better results given by this species in the MV calibration. Results of the global model are acceptable, as can be observed by the correlation coefficient (Table 4) and biplots between predicted and observed values (Fig. 6), but it requires the definition of three intervals dependent on the precipitation intensity.

These results point out the need of considering precipitation in biomonitoring the atmospheric deposition of saline elements, in a way that the relationship between precipitation and lichen accumulation of saline elements can be described. This is important not only for calibration purposes, but also for time modelling of airborne deposition of marine elements, since it was recognised that precipitation plays an important role in the spatio-temporal estimation of salt levels in lichens (Figueira et al., 2001). For other elements, like Cu, where it was found that uptake by lichen transplants is promoted by rain (Branquinho, 1997), leading to the inclusion of this factor in the calibration model between lichen and dust gauges concentrations (Pereira et al., 1995).

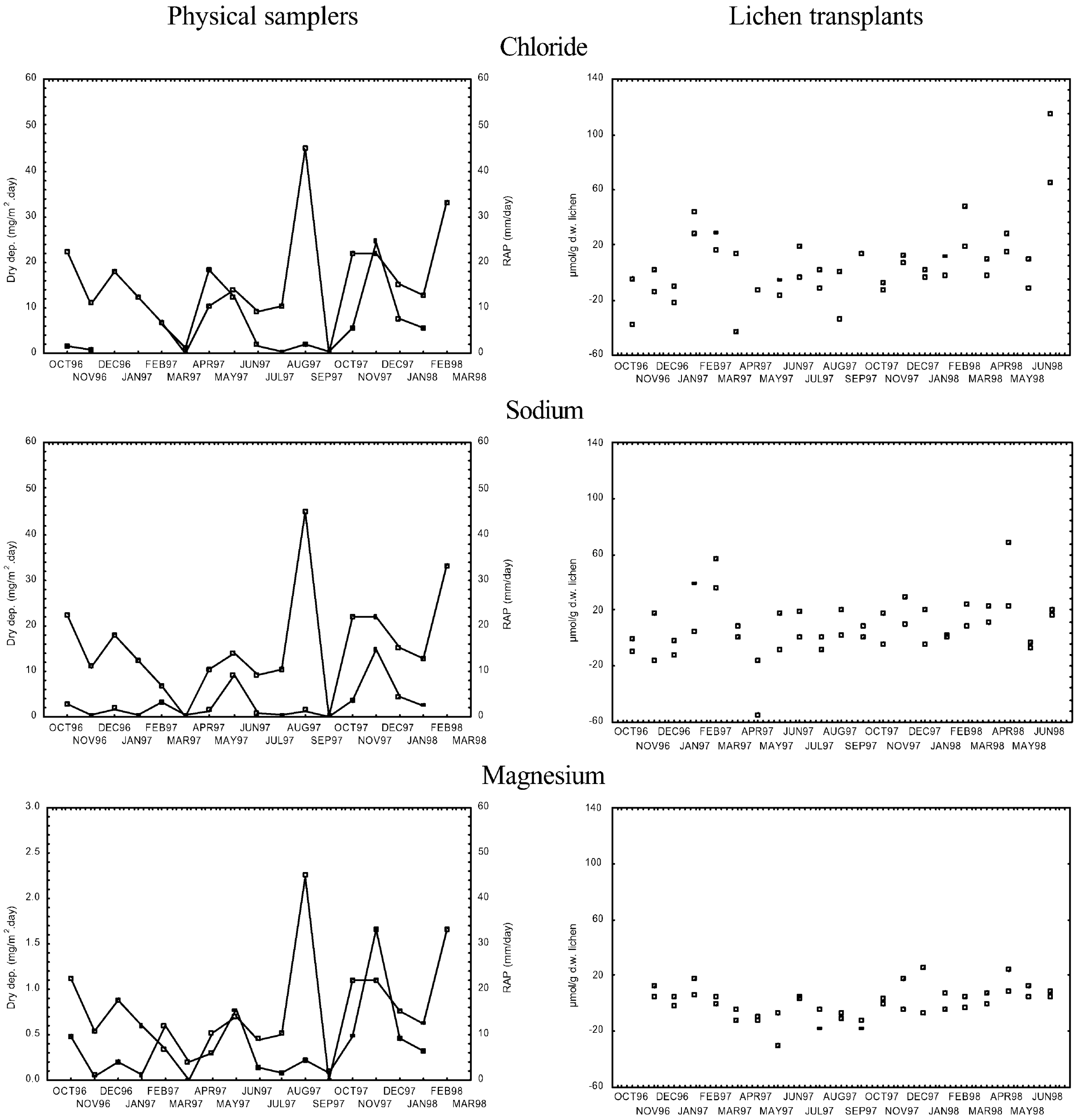

Fig. 4. Time profiles of physical (left) and biological (right) data obtained at ME station. In physical-data patterns, solid symbols are for dry deposition and open ones are for RAP. In biological-data patterns, solid symbols are for *Usnea* spp. and open ones are for *Ramalina calicaris*.

The understanding of the effect of precipitation in the uptake efficiency of elements by lichens is of paramount importance. Its influence in uptake or release of ions cannot be assumed to show a linear behaviour through all the concentration ranges that biomonitors are exposed to in nature. It is much dependent on the affinity of the element to the exchange sites of the cell wall, and the form that the ion is presented to them (soluble or particles). In addition to this, lichen morphology is likely to play an important role in retention or leaching of elements. In studies where the temporal variability is important, there is, therefore, the need to identify the influence of precipitation in element uptake by biomonitors.

The wet deposition could not be related to the lichen uptake of saline elements, because no significant correlation coefficients could be found. The washout effect of precipitation seems to superimpose the ability of the lichens to retain elements. This can be due to the low affinity of the saline elements to the exchange sites, which is one of the reasons why only 20% of extracellular soluble sodium ions are bound to the cell wall exchange sites (Figueira et al., 1999c). For elements with high affinity to the exchange sites (e.g. Pb), it was

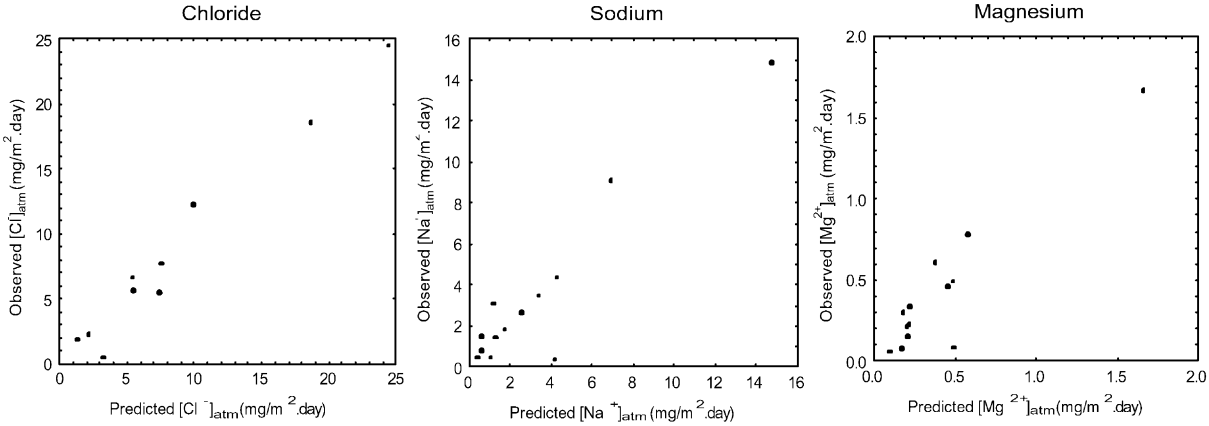

Fig. 5. Results of the calibration model of *Ramalina calicaris* for Cl^-, Na^+ and Mg^{2+} at the ME Station.

Table 3
Regression coefficients (a_i, b_i) and correlation coefficients (r) of the calibration model for lichen species in ME station

	a_0	a_1	a_2	a_3	a_4	b_0	b_1	b_2	b_3	b_4	r
Ramalina calicaris											
Chloride	−11.07	0.845	−0.217	−0.699	2.018	0.29	0.996	0.017	0.182	0.831	0.98
Sodium	−5.97	−0.038	0.098	−0.264	0.810	30.00	−0.722	1.866	−0.144	−1.309	0.94
Magnesium	0.00	−0.002	0.009	−0.026	0.031	3.01	−0.065	0.184	0.004	−0.121	0.93
Usnea spp.											
Chloride	15.65	−0.372	0.090	−0.147	−0.841	0.29	0.404	1.502	−0.484	−0.735	0.86
Sodium	−4.74	−0.063	0.107	−0.145	0.497	33.57	1.097	0.116	−0.762	−0.968	0.99
Magnesium	0.09	−0.005	0.010	−0.014	0.004	3.83	0.118	0.020	−0.091	−0.113	0.96

Table 4
Regression coefficients (a_i b_i, c_i) and correlation coefficients (r) of the global calibration model for *Ramalina calicaris* in the MV and ME stations

	Chloride	Sodium	Magnesium
a_0	2.316	−1.635	0.315
a_1	−0.006	0.025	−0.002
a_2	0.017	0.038	0.002
a_3	0.033	−0.070	0.003
a_4	0.266	1.508	0.006
b_0	2.496	2.136	0.232
b_1	0.041	0.014	0.001
b_2	−0.025	−0.002	0.001
b_3	−0.046	−0.031	−0.004
b_4	0.278	0.021	0.006
c_0	0.271	30.004	3.011
c_1	0.997	−0.722	−0.065
c_2	0.016	1.866	0.184
c_3	0.182	−0.144	0.004
c_4	0.833	−1.309	−0.121
r	0.77	0.73	0.86

observed that precipitation could improve the uptake by biomonitors (Groet, 1976; Pott and Turpin, 1998), either because it makes more ions available from wet deposition, or it promotes the formation of ions from particles previously deposited on the biomonitor.

4. Conclusions

The accumulation of saline elements by lichens is influenced by precipitation. The principal effect of this factor seems to be to washout or leach surface elements from lichens. It depends, however, on the rain intensity, since it was not possible to describe the runoff effect for several precipitation ranges using a single linear model. In order to calibrate lichen monitoring of saline dry deposition against physico-chemical samplers, a multivariate model was built, using a stepwise approach. The model predicts the amount of dry deposition of a saline element from the concentration of saline elements on lichen and the intensity of rain, after establishing breakpoints based on the last variable. It was applied for two stations with different levels of annual precipitation, by selecting specific breakpoints for each situation. The fitting of the model showed good results for both stations and a global calibration model was applied using the breakpoints determined for each station. In order to understand the biological and ecological meaning of the breakpoints, more research is needed which can explore the importance of factors like the ionic affinity of the element to exchange sites of lichen's cell wall, the importance of morphology in particle and water retention, among other factors.

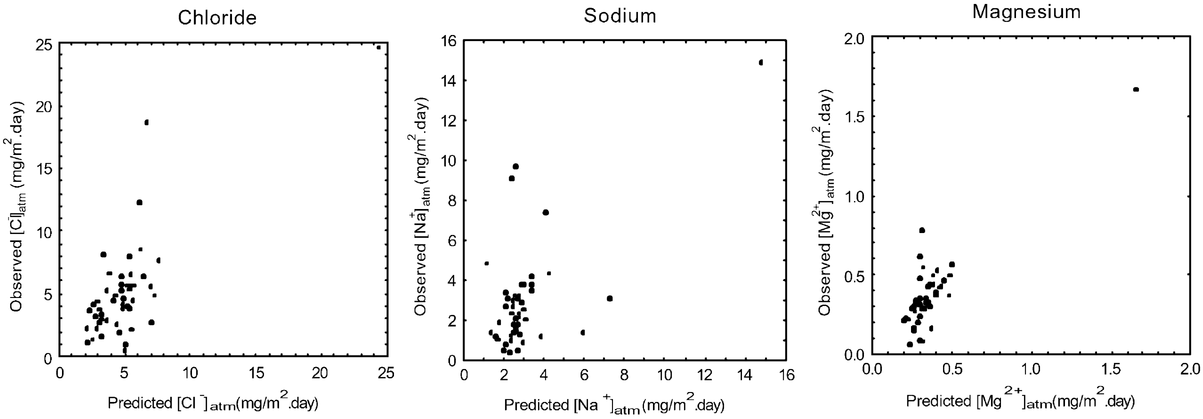

Fig. 6. Results of the global calibration model obtained for *Ramalina calicaris* at both stations (MV, ME).

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References

Beverland, I.J., Crowther, J.M., 1992. On the interpretation of event and sub-event rainfall chemistry. Environmental Pollution 75, 163–174.

Boonpragob, K., Nash III., T.H., Fox, C.A., 1989. Seasonal deposition patterns of acidic ions and ammonium to the lichen *Ramalina menziesii* Tayl in Southern California. Environmental and Experimental Botany 29, 187–197.

Branquinho, C., 1997. Improving the use of lichens as biomonitors of atmospheric metal pollution, PhD thesis, Faculty of Sciences, University of Lisbon, 150 pp.

Branquinho, C., Brown, D.H., Catarino, F., 1997. The cellular location of Cu in lichens and its effects on membrane integrity and cholophyll fluorescence. Environmental and Experimental Botany 38, 165–179.

Brown, D.H., Wells, J.M., 1988. Sequential elution technique for determining the cellular location of cations. In: Glime, J.M. (Ed.), Methods in Bryology. Hattori Botanical Laboratory, Nichinan, pp. 227–233.

Brown, D.H., Brown, R.M., 1991. Mineral cycling and lichens: the physiological basis. Lichenologist 23, 293–307.

Brown, D.H., Buck, G.W., 1985. The cellular location of metals in two bryophytes and a lichen. Cryptogamie, Bryol. Lichénol. 6, 279–286.

Figueira, R., Branquinho, C., Pacheco, A.M.G., Sousa, A.J., Catarino, F. 1994. Desenvolvimento de biomonitores de salinidade atmosférica. Actas da *4a Conferência Nacional sobre a Qualidade do Ambiente (Lisboa)*, vol. II, M36-M48.

Figueira, R., Catarino, F., Pacheco, A.M.G., Sousa, A.J., 1998. *In situ* studies on sea-salt uptake by epiphytic lichens. Sauteria 9, 143–150.

Figueira, R., Sousa, A.J., Pacheco, A.M.G., Catarino, F., 1999a. Saline variability at ground level after kriging data from *Ramalina* spp. Biomonitors. The Science of the Total Environment 232, 3–11.

Figueira, R., Sousa, A.J., Brown, D.H., Catarino, F., Pacheco, A.M.G., 1999c. Natural levels of saline elements in lichens: determination of cellular fractions and their importance as saline tracers. Lichenologist 31, 183–196.

Figueira, R., Sousa, A.J., Pacheco, A.M.G., Catarino, F., 1999b. Variability of sea-salt deposition assessed by lichen monitoring in the south-west Portuguese coast. In: Brebbia, C.A., Jacobson, M., Power, H. (Eds.), Air Pollution VII. WIT Press, Southampton, pp. 599–607.

Figueira, R., Sousa, A.J., Pacheco, A.M.G., Catarino, F., 2001. Use of secondary information in space-time statistics for biomonitoring studies of saline deposition. Environmetrics 12, 203–217.

Garty, J., Perry, A.S., Mozel, J., 1982. Accumulation pf polychlorinated biphenyls (PCBs) in the transplanted lichen *Ramalina duriaei* in air quality biomonitoring experiments. Nordic Journal of Botany 2, 583–586.

Groet, S.S., 1976. Regional and local variations in heavy metal concentrations of bryophytes in the Northeastern United States. Oikos 27, 445–456.

Jeran, Z., Byrne, A.R., Batic, F., 1995. Transplanted epiphytic lichens as biomonitors of air-contamination by natural radionuclides around the Zirovski Vrh uranium mine, Slovenia. Lichenologist 27, 375–385.

Kytöviita, M.-M., Crittenden, P.D., 1994. Effects of simulated acid rain on nitrogenase activity (acetylene reduction) in the lichen *Stereocaulon paschale* (L.) Hoffm., with special reference to nutritional aspects. New Phytologist 128, 263–271.

Loppi, S., Pacioni, G., Olivieri, N., Di Giacomo, F., 1998. Accumulation of trace metals in the lichen *Evernia prunastri* transplanted at biomonitoring sites in central Italy. Bryologist 101, 451–454.

Nash III., T.H., 1996. Nutrients, elemental accumulation and mineral cycling. In: Nash III., T.H. (Ed.), Lichen Biology. Cambridge University Press, Cambridge, pp. 136–153.

Nieboer, E., Richardson, D.H.S., 1980. The replacement of the nondescript term 'Heavy Metals' by a biologically and chemically signification of metal ions. Environmental Pollution 1, 3–26.

Pacheco, A.M.G., Sousa, A.J., Figueira, R., Branquinho, C., Catarino, F., 1995. Salinidade atmosférica: um vector de risco natural. Técnica 1/95, 29–33.

Pereira, M.J., Branquinho, C., Soares, A., 1995. Modelling air pollution in a copper mine by using physical and biological monitoring. In: Power, H., Moussiopoulos, N., Brebbia, C.A. (Eds.), Proceedings of the Third International Conference on Air Polution. Air Pollution Engineering and Management, Porto Carras, Greece, pp. 49–57.

Pilegaard, K., 1979. Heavy metals in bulk precipitation and transplanted *Hypogymnia physodes* and *Dicranoweisia cirrata* in the vicinity of a danish steelworks. Water, Air, and Soil Pollution 11, 77–91.

Pott, U., Turpin, D.H., 1998. Assessment of atmospheric heavy metals by moss monitoring with *Isothecium stoloniferum* Brid. in the Fraser Valley, B.C., Canada. Water, Air, and Soil Pollution 101, 25–44.

Rühling, Å., Steiness, E. (Eds.), 1998. *Atmospheric heavy metal deposition in Europe 1995–1996*. NORD 1998:15, Copenhagen, 66 pp.

Schales, O., Schales, S.S., 1941. A simple and accurate method for the determination of chloride in biological fluids. Journal of Biological Chemistry 140, 879–884.