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Lichen-Transplant Biomonitoring in the Assessment of Dispersion of Atmospheric Trace-Element Pollutants: Effects of Orientation Towards the Wind Direction

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Abstract. Transplants of the epiphytic lichen *Parmelia sulcata* were suspended in nylon bags in a special device allowing a fixed orientation of the lichen towards the wind direction, viz.facing the wind (F orientation) or shielded from the wind by its substrate (T orientation). The F- and T-orientation data were mutually compared and also with reference values (=time zero values of the transplants). The data were analysed via Monte Carlo added target transformation factor analysis (MCATTFA) to get information on possible emission-source profiles and their contributions to total-element levels in transplants, in F- and T-orientations. Both orientations did not differ for Na, Mg, P, Cl, K, Fe, Co, Ni, Ga, Se, Br, Sr, Ba, La, Nd, Sm, Lu and Ta, but showed some time-related differences for Cr and Zn. For the remaining elements the data presents a high variability. Under the conditions of the experiment, F- and T-oriented transplants generally did not result in differences in source profiles reflected, nor in differences in source contributions to element levels in the transplants.

Key words: facing-wind, lichens, multi-element, opposing-wind, transplants

1. Introduction

Lichens are slowly growing symbiotic associations of fungi and green algae, which depend mainly on atmospheric input of mineral nutrients (Nash III, 1996). This fact makes them one of the best bioindicators of air pollution (Garty, 2001). Under natural conditions epiphytic lichens are distributed all around the circumference of tree stems or branches or preferentially in specific positions. They may or may not be shielded by stems or branches from wind or rain; they may grow horizontally, vertically or in any other direction. Regardless of this variability, or to rule out any specific influences, in many surveys lichen sampling is from all around the tree and/or from all positions (Mulgrew *et al.*, 2000). With lichen transplants, the material is generally positioned without any pre-set fixed position (Bargagli, 1998).

Usually the position of the lichen is not taken into account as a variable of importance. In the period 1994–1996, an atmospheric exposure experiment with lichen transplants was held in Portugal using six sampling locations from north to south of Portugal. The results indicated that positioning of the transplants towards the wind direction was an important factor for the eventual interpretation (Reis, 2001). The experiment further showed that the amount of direct rain was an important factor to be accounted for the interpretation of the data. Moreover, another previous lichen-based survey, held in 1993, showed that the Setúbal area is quite polluted (Freitas *et al.*, 2000). The present paper focuses on transplant positioning in biomonitoring of trace-element air pollution, in an exposure experiment performed in the Setúbal area. It addresses in particular the orientation of lichen transplants towards the wind direction, both in terms of total-element concentrations, time of exposure, and transplant expression of possible emission-source profiles.

2. Materials and Methods

2.1. SAMPLING AND SAMPLE HANDLING

Samples of the epiphytic lichen *Parmelia sulcata* were collected from olive trees at a height of about 1.5 m in areas considered free from pollution (Freitas *et al.*, 2000). Samples of about 2–3 g (lichen with substrate attached) were put into nylon net bags and suspended also at a height of about 1.5 m at locations in the Sado estuary region situated 50 km south from Lisbon, Portugal. The exposure area comprised a rectangle of 15 km $\times$ 25 km where a 2.5 km $\times$ 2.5 km grid was used (Costa *et al.*, 2002). Two sets of four transplants each were set out in 47 places comprising (i) a polyethylene cover to prevent direct rain, (ii) a set-up to fix the lichen transplant in a system which rotates according to the wind direction, and (iii) a fixed orientation of the lichen, either facing the wind (F orientation) or shielded from the wind by its substrate (T orientation) (Reis *et al.*, 1999). The transplants were recovered after 3, 6 and 9 months of exposure. In the laboratory, transplants were clean, washed for 30 s in de-ionized water, freeze-dried, and ground (for about 5 min at 1500 rpm) in a Teflon mill.

2.2. ANALYSIS VIA INAA AND PIXE

The samples were analysed by instrumental neutron-activation analysis (INAA) and proton-induced X-ray emission (PIXE) for a range of elements. For INAA pellets of 500 mg were irradiated at the Portuguese nuclear reactor (RPI) at Instituto Tecnológico e Nuclear (ITN) in Portugal and measured with a high-purity germanium detector. Calibration was performed by the k_0 factor method. For PIXE analysis a pellet of a thin layer of lichen powder in a boric acid support was made. Samples were irradiated with the van de Graaff accelerator at ITN. The X-ray spectra were measured with a Si(Li) detector. Quality control was pursued by the analysis of IAEA-336 lichen and CTA-OTL-1 oriental tobacco leaves reference materials.

2.3. DATA HANDLING

F-tests were applied to the data to test the significance of the differences between element levels of the exposed transplant and the reference levels (RL), consisting of the lichen element content at time zero. Student t-tests were used to test the significance of differences between the two ways of orientation, towards the wind direction (F for facing the wind, T for being shielded from the wind by their substrates). Monte Carlo assisted target transformation factor analysis (MCATTFA; Kuik *et al.*, 1993a,b) was applied to the data at IRI (Delft University of Technology, The Netherlands) to study F- and T-related possible emission-source profiles and their respective contributions to F- and T-associated total-element contents. For MCATTFA, 10 elements were selected: Na, Cl, Ca, Sc, V, Fe, Ni, Cu, As, and Pb. Contour plots (Surfer, Golden Software Inc) of transplants ratio (facing/opposing the wind) were performed. These plots are based on a $1/r^3$ extinction rule. Data on the predominant wind direction during the exposure period were obtained from electricity of Portugal (EDP/CPPE) monitoring site at the fuel power station perimeter (south of the grid).

3. Results

Table I presents statistics on the element data obtained for 3, 6 and 9 months, respectively, for wind facing (F) and wind-shielded (T) transplants in Sado estuary region. The probability of F = T was determined (using a t-test) by calculating the ratio of F- and T-data variances. Whenever the ratio of F- and T-data variances leads to a probability of a different variance below 95%, the probability of an equal mean value was calculated assuming equal variances. The designation "different" means that the variances are different with a certainty larger than 95% and the t-test can not be applied, only approximate procedures can be used (Woolson, 1987) which was not the case. The analytical approach (INAA or PIXE) for elements determined by both methods was selected following Freitas *et al*. (2000) and Marques *et al*. (2003).

3.1. F AND T DIFFERENCES FROM RL

For each element the probability that F $\neq$ RL or T $\neq$ RL was calculated. Using a 95% probability threshold, results can be summarised as follows.

3.1.1. *Equal Behaviour of F and T towards RL*

- Different from RL after 3, 6 and 9 months of exposure: Na, S, Cl, V, Mn, Ni, Cu, Zn, As, Se, Br, Sr, and Sb.
- Not different from RL after 3, 6 and 9 months of exposure: P, K, Sc, Fe, La, Ce, Nd, Sm, Eu, Tb, and Th.

Table I. Statistics on the element data obtained for 3, 6 and 9 months of exposure and for wind facing (F) and wind-shielded (T) transplants in the Sado-estuary region. For each element the probability of F ≠ RL or T ≠ RL was calculated using an F-test (values ≥ 0.95 are marked in grey). The probability of F = T was calculated using a *t*-test (whenever F and T present a probability higher than 0.95 of having different variances, F and T are considered "different", being marked grey)

	Probability of being different from RL								
	3 months		6 months		9 months		Probability of F = T		
Elements	F	T	F	T	F	T	3 months	6 months	9 months
Na	1.000	1.000	1.000	1.000	1.000	1.000	0.882	0.828	0.873
Mg	0.956	0.986	0.791	0.922	1.000	0.991	0.957	0.674	0.817
Al	0.392	0.905	0.996	0.775	0.891	0.720	0.896	Different	0.750
Si	0.793	0.997	1.000	0.991	0.998	0.963	Different	Different	0.759
P	0.698	0.933	0.487	0.049	0.109	0.101	0.811	0.926	0.876
S	1.000	1.000	1.000	1.000	1.000	1.000	0.874	0.671	Different
Cl	1.000	1.000	1.000	1.000	1.000	1.000	0.799	0.948	0.849
K	0.815	0.892	0.394	0.243	0.919	0.556	0.788	0.556	0.882
Ca	1.000	0.575	0.998	0.688	1.000	1.000	Different	Different	0.874
Sc	0.830	0.013	0.504	0.403	0.229	0.274	Different	0.853	0.705
Ti	0.692	0.996	1.000	0.914	0.997	0.981	Different	Different	0.757
V	0.997	0.949	1.000	1.000	1.000	1.000	0.895	Different	0.991
Cr	0.999	0.798	1.000	0.995	0.972	1.000	Different	Different	Different
Mn	1.000	1.000	1.000	1.000	1.000	1.000	0.852	Different	0.763
Fe	0.706	0.103	0.444	0.628	0.629	0.518	0.753	0.844	0.710
Co	0.403	0.344	0.817	0.918	1.000	0.997	0.880	0.831	0.713
Ni	1.000	1.000	1.000	1.000	1.000	1.000	0.987	0.700	0.828
Cu	1.000	1.000	1.000	1.000	1.000	1.000	Different	0.866	0.922
Zn	1.000	1.000	1.000	1.000	1.000	1.000	0.973	Different	Different
Ga							0.890	0.980	0.954
As	1.000	1.000	1.000	1.000	1.000	1.000	Different	0.818	0.955
Se	0.986	0.997	0.999	1.000	1.000	1.000	0.518	0.755	0.739
Br	1.000	1.000	1.000	1.000	1.000	1.000	0.864	0.782	0.712
Rb	0.024	0.535	0.818	0.118	0.402	0.541	Different	0.950	0.790
Sr	1.000	1.000	1.000	1.000	1.000	1.000	0.987	0.677	0.699
Zr	1.000	1.000	0.939	0.773	1.000	1.000	0.996	0.747	Different
Sb	1.000	1.000	1.000	1.000	1.000	1.000	0.947	0.816	Different
Ba	0.400	0.286	0.836	0.898	0.999	0.995	0.738	0.792	0.695
La	0.857	0.553	0.329	0.281	0.497	0.075	0.758	0.899	0.715
Ce	0.469	0.731	0.079	0.488	0.903	0.691	Different	0.913	0.652
Nd	0.071	0.684	0.769	0.902	0.877	0.0925	0.774	0.942	0.986

(*Continued on next page*)

Table I. (*Continued*)

	Probability of being different from RL								
	3 months		6 months		9 months		Probability of F = T		
Elements	F	T	F	T	F	T	3 months	6 months	9 months
Sm	0.100	0.698	0.956	0.549	0.812	0.769	0.600	0.971	0.998
Eu	0.812	0.068	0.130	0.532	0.690	0.391	Different	0.846	Different
Tb	0.636	0.433	0.386	0.819	0.513	0.847	Different	0.984	0.767
Lu	0.558	0.104	0.170	0.530	0.868	0.118	0.597	0.775	0.704
Hf	0.661	0.685	0.346	0.811	0.698	0.833	Different	Different	0.710
Ta	0.717	0.039	0.389	0.419	0.521	0.328	0.702	0.833	0.671
Hg	0.865	0.406	0.374	0.412	1.000	0.899	0.650	0.916	Different
Pb	0.848	0.495	0.998	0.999	1.000	1.000	0.964	0.781	Different
Th	0.526	0.752	0.167	0.873	0.839	0.673	Different	0.922	0.729
U	1.000	0.310	1.000	0.873	1.000	0.999	Different	Different	0.789

- Different from RL for a certain exposure period: Mg (3 and 9 months), Co (9 months), Zr (3 and 9 months), Ba (9 months), and Pb (6 and 9 months).

3.1.2. *Differences in Behaviour of F and T towards RL*

- In the F-set Ca and U differ from RL at 3, 6 and 9 months of exposure; for the T set this occurs at 9 months of exposure.
- In the F-set Si differs from RL at 6 and 9 months of exposure; for T set this occurs at 3, 6 and 9 months of exposure.
- In the F-set Cr differs from RL at 3, 6 and 9 months of exposure; for the T sets this occurs at 6 and 9 months of exposure.
- In the F-set Hg differs from RL at 9 months of exposure; T set does not differ from RL.

These observations indicate that with a few exceptions: (i) the wind direction does not significantly affect the bioaccumulation of elements into *Parmelia sulcata*; (ii) lithophilic elements did not accumulate during the exposure, for both F- and T-orientation; (iii) both F- and T-oriented transplants are sensitive enough to indicate trace-element pollutants; (iv) physiological elements are not lost through the exposure for both F- and T-orientation. The latter observation serves to underline the absence of any visible toxicity action: losses of K and P are regarded as indicating cell-membrane damage (Nash, 1996). Based on Table I either F- or T-oriented transplants can be regarded as accumulating trace-element pollutants, in the present experiment without changes in physiological guide elements and

without accumulating soil-related elements. Some time-related differences in behaviour between F- and T-orientation were observed for Si, Ca, Cr, Hg and U, which might indicate F- and T-related differences in dynamics, but for the majority of elements F- and T-orientations behave similarly towards RL.

3.2. DIFFERENCES BETWEEN F- AND T-ORIENTED TRANSPLANTS

Table I also presents statistics on the possible differences between F- and T-orientations of the transplants. This data may be summarised as follows: F- and T-orientations are significantly different for 3, 6 and 9 months exposure (Cr), for 3 and 6 months exposure (Si, Ca, Ti, Hf and U), for 6 and 9 months exposure (Zn), for 3 and 9 months exposure (Eu), for 3 months exposure (Sc, Cu, As, Rb, Ce, Tb and Th), for 6 months exposure (Al, V and Mn) and for 9 months exposure (S, Sb, Zr, Hg and Pb). F and T are not significantly different (all exposure periods) for Na, Mg, P, Cl, K, Fe, Co, Ni, Ga, Se, Br, Sr, Ba, La, Nd, Sm, Lu and Ta.

General conclusions may be that the transplants behave similarly for Na, Mg, P, Cl, K, Fe, Co, Ni, Ga, Se, Br, Sr, Ba, La, Nd, Sm, Lu and Ta and differently for Cr and Zn, while the elements Si, Ca, Ti, Hf and U show a behaviour that lies in between.

3.3. F AND T ORIENTATION IN REFLECTING EMISSION-SOURCE PROFILES

MCATTFA was used to compare data from F- and T-orientations. The comparison was made for the common source profiles, both in time relations, and in terms of the relative source contributions to total-element levels. Due to loss of some systems, 39 F-transplants (F3) and 39 T-transplants (T3) were removed after 3 months, 34 F-transplants (F6) and 35 T-transplants (T6) after 6 months and after 9 months exposure 25 F-transplants (F9) and 31 T-transplants (T9) were collected (Marques *et al.*, 2004). Since a few sampling sites were available per set, MCATTFA analyses ran with a selection of only10 elements (Na, Cl, Ca, Sc, V, Fe, Ni, Cu, As, and Pb) following Henry (1991) in his reasoning towards a rule-of-thumb relationship between number of sampling sites and number of elements to be taken into the factor analysis. The approach to determine the number of factors was based on the selection of common factors in F- and T-orientations, and further essentially followed Kuik *et al.* (1993a,b). Source profiles (factor loadings, not shown) could be associated to *industrial emissions* (Cu, Pb, Ni and V associated elements), *sea-salt spray* (Na and Cl associated elements), *soil* (Sc and Fe associated elements) and *agricultural activities* (As and Ca associated elements). These factor interpretations largely follow Nriagu (1989), who showed V, Ni, Pb and Cu as highly correlated with oil combustion, with industrial emissions especially (Rahn *et al.*, 1999). Na and Cl are the elements more correlated to sea salt spray (Bowen, 1979). The As, Ca factor could be related to a pesticide- or herbicide-associated use of As in vineyards (NORD 1987:21, NORD 1994:9), in combination with the use of

Table II. Comparison in relative factor contributions in F- and T-oriented lichen transplants[a]. All factor contributions in F–T comparisons with slopes with P values < 0.05 should be considered as non-identical. The table only shows the $P > 0.05$ values

	P values			
	T3-1	T3-2	T3-3	
F3-1	0.053			
F3-2		0.98		
F3-3			0.088	
F3-4				
	T6-1	T6-2	T6-3	T6-4
F6-1				
F6-2		0.31		
F6-3				
F6-4				0.33
	T9-1	T9-2	T9-3	T9-4
F9-1	0.17			
F9-2			0.55	0.73
F9-3		0.11		
F9-4				0.089

[a]Relative contributions obtained for F- and T orientations after initial normalisation of factor-explained total variances per element to 100 %. In the table, the Fa-b or Ta-b notation indicates F- or T-oriented transplants, the character "a" denotes the months of exposure and the character "b" the bth source factor; thus, F3-1 means the F transplant after 3 months of exposure and the 1st source factor. In MCTTFA, identical factors are recognised by the calculation of so-called "factor-conflicts" (Kuik *et al.* 1993a, b), but it should be noted here that common factors do not need to carry the same factor number. *Industrial emissions:* F3-1, T3-1, F6-3, T6-1, F9-1 and T9-1, *sea-salt spray:* F3-2, T3-2, F6-2, T6-2, F9-3 and T9-2, *soil:* F3-3, T3-3, F6-1, T6-3, F9-2 and T9-3 and *agricultural activities:* F3-4, F6-4, T6-4, F9-4 and T9-4. In the calculations the contribution vectors were compared by calculating the slopes between two outcomes: unit slope values indicate identical contributions.

arsenous anhydride and sodium arsenite in cuts-protection when vineyards are trimmed (www.dgpc.min-agricultura.pt/), and the use of mixed Ca_2OH and $CuSO_4$ in control of vineyard diseases.

Average contributions of common factors (source profiles) to total-element occurrences in F- and T-oriented transplants were calculated and compared. Table II gives data on these comparisons: factor contributions were calculated for both F- and T-oriented transplants, for each exposure period. The data in Table II indicate that, apart from difficulties in interpreting two factor contributions in the

6 months of exposure, and a double significance occurrence for the F-T comparison in the 9-month exposure, in most cases F- and T-oriented transplants show comparable source contributions over all exposure periods considered. Nevertheless, this is not the case of the industrial and soil factors in the 6 months data.

Apart from the data above, which predict that F- and T-oriented transplants do not differ in factor contributions, the initial data were interpreted also in terms of time relations. Here, factor contributions were compared between exposure periods, to judge shifts in relative source importance with time. Table III gives results for 3 and 6-, 6 and 9-, and 3 and 9-month exposure periods comparisons for both F- and T-oriented transplants, and generally indicates that relative source contributions do not change in time, although there are some exceptions. It is stressed that, although in general no shifts in time relations are observed, some elements do present a higher accumulation sufficient enough to produce a change in the factor contribution of related factors. For instance, F3-2 and F9-3 in Table III, both related to sea-salt spray, were considered non-identical. The sampling area is next to the sea and it is well known that sodium and chlorine are elements easily accumulated and also easily leached from lichens (Figueira, 2002). A high accumulation rate and no leaching effects (by the use of a proper exposure device equipped with a "hat") are expected for the sea-related elements. For facing-the-wind transplants (F) this has led to a shift in element contents observed between 3 and 9 months of exposure for the sea factor.

4. Discussion and Final Remarks

Generally reviewing the present results, the data indicate that F- and T-oriented transplants do not differ regarding the accumulation of Na, Mg, P, Cl, K, Fe, Co, Ni, Ga, Se, Br, Sr, Ba, La, Nd, Sm, Lu and Ta, regardless of the exposure periods examined. Differences between F- and T-oriented transplants were obtained for Cr and Zn. For the remaining elements, a high variability is observed. This observation answers a hypothesis raised to explain the differences between replicates data found by Reis (2001), who reasoned that both orientation and precipitation were influencing accumulation results. Although the present results show consistent data for a large variety of elements, one may reason that differences between study set-ups may have caused some differentiation in transplant behaviour. The present study implied transplant protection against direct rain, and it was of different length of the exposure periods, and the location of the sampling sites.

According to Nriagu (1989), Cr and Zn are the elements more associated with iron and steel. Marques *et al.* (2004), in applying MCATTFA to the Sado estuary data using 26 elements (both F- and T-oriented sets), found Cr and Zn as associated with factors attributed to oil combustion, sea-salt spray and iron and steel processing and handling. Contour plots were made for Cr and Zn in transplants ratio (F/T positioning) values (Figure 1). For Cr, after 3 months of exposure, F over T ratio was

Table III. Comparison in shifts with exposure periods relative to factor-contributions in F- and T-transplants[a]. P values > 0.05 (shown) indicate unit slope values. All factor contributions in F–T comparisons with slopes with $P < 0.05$ values should be considered as non-identical. The table only shows $P > 0.05$ values. For explanation of notations and source profiles, see Table II

	P Values			
	F3-1	F3-2	F3-3	F3-4
F6-1				
F6-2		0.74		
F6-3	0.14			
F6-4				0.17
	F6-1	F6-2	F6-3	F6-4
F9-1	0.17			
F9-2				
F9-3		0.069		
F9-4		0.082		0.38
	F3-1	F3-2	F3-3	F3-4
F9-1	0.59			
F9-2				
F9-3				
F9-4				0.052
	T3-1	T3-2	T3-3	
T6-1				
T6-2		0.29		
T6-3			0.14	
T6-4				
	T6-1	T6-2	T6-3	T6-4
T9-1	0.33			
T9-2		0.10		
T9-3				
T9-4	0.27			
	T3-1	T3-2	T3-3	
T9-1	0.20			
T9-2		0.068		
T9-3				
T9-4	0.37		0.56	

[a]In the calculations the contribution-vectors were compared by calculating the slopes between two outcomes; an unit slope values indicate identical contributions.

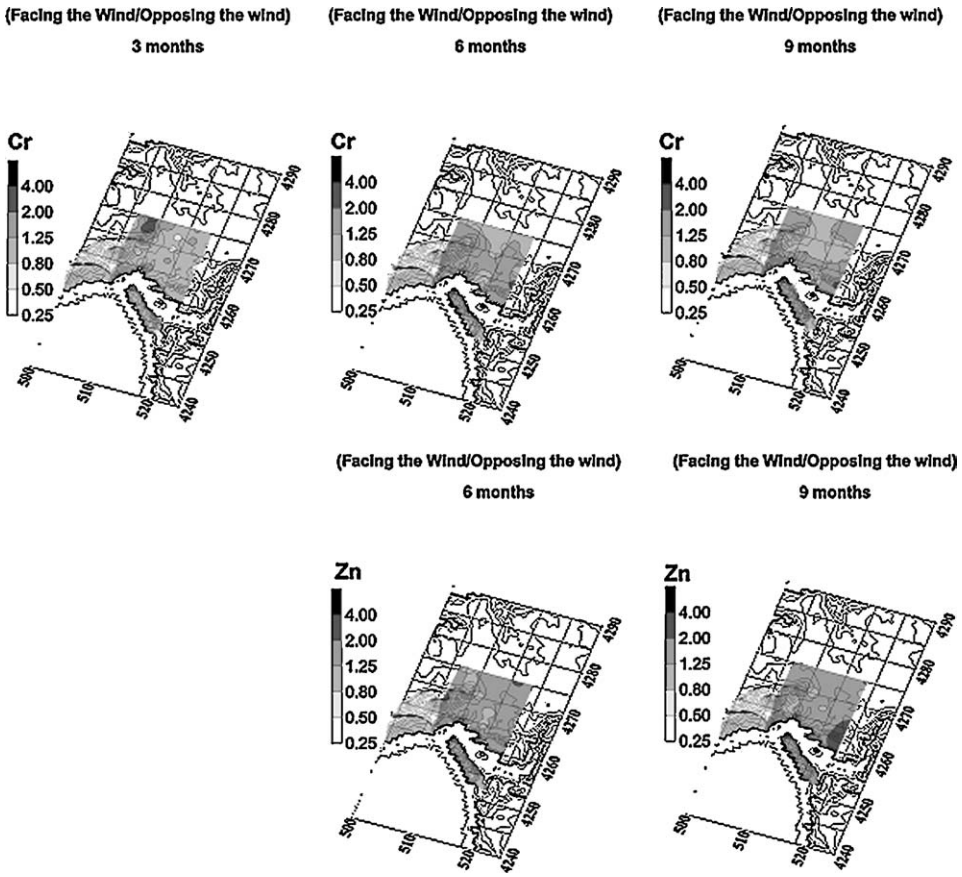

Figure 1. Contour plots (Surfer, Golden Software Inc) of the transplant ratio F/T (facing/opposing the wind) for Cr (3, 6 and 9 months of exposure) and Zn (6 and 9 months of exposure). These plots are based on a $1/r^3$ extinction rule.

higher than 3 in the west upper part of the grid. After 6 months, the patterns were shifted into another differentiation, which remained still identifiable at 9 months, in the eastern part of the grid (F/T between 2 and 3). For Zn, after 6 months of exposure, small F/T differentiation appeared in the North, Northwest and Southeast (F/T between 2 and 3). At 9 months the F/T differentiation persisted only at the Southeast part of the grid. The wind direction during the first 3 months of exposure was more diffuse and at 6 and 9 months of exposure was coming mostly from the north (not shown here). Here it should be noted that concentrations may change with changing wind directions but no straightforward changes in F/T ratios are to be expected.

Concluding, for the majority of elements determined, the data indicate the absence of any significant difference in the behaviour of F- and T-oriented transplants. Transplants in both orientations accumulate elements to similar levels,

and they both don't show any appreciable accumulation of lithophilic elements. Moreover, F- and T-oriented transplants both don't show appreciable losses of K and P, and in general they show similar source profiles in MCATTFA approaches and similar outcomes with respect to calculated source contributions to total-element concentrations, although some exceptions are observed. Uptake and release processes of elements in lichens are mechanisms still not very well known, especially in field conditions (Wolterbeek *et al.*, 2002) and more work should be done in this area in order to be able to fully understand and explain the differences found.

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