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Parmelia sulcata lichen transplants positioning towards wind direction (Part II): element concentrations and relationships with atmospheric element deposition

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Abstract *Parmelia sulcata* transplants were positioned in three different exposure systems allowing three different influxes: free influx (Fi), horizontal influx (Hi) and vertical influx (Vi). Results suggest the absence of any wind-directional effects on element accumulation within Fi and Hi systems, but underline that differences in response may be observed in relation with the transplant set-up systems. The Vi system generally shows poor results, while the performance of the Hi and Fi systems depends on the element involved. Results were obtained for a specific lichen, and therefore are not necessarily representative for other lichens.

Keywords Lichen transplants · Trace elements · Wind influxes · Transplant hanging systems

Introduction

Under natural conditions, epiphytic lichens are either distributed all around the circumference of tree stems or branches, or distributed 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 positioning. Regardless of this variability, or to rule out any specific influences, sampling is generally from all around the tree, although some investigators prefer other methodologies [1]. In air pollution studies using lichen transplants, the material is generally positioned without any pre-set fixed position [2]. Thus, in both cases (sampling and exposure), positioning of the lichen, is not usually taken into account as a variable of importance. The present study comprises three transplant-positioning approaches; free, horizontal covering, and vertical covering. Furthermore, in a number of these set-ups, wind directional positioning (facing or shielded) was addressed. The results, which are considered as of importance for both transplant- and in situ approaches in surveys, should give further insights in the necessary protocols for lichen biomonitoring of trace-element air pollution.

Experimental

Exposition set-ups and sampling

Parmelia sulcata was collected from a region of Portugal considered clean from the point of view of air pollution [3]. Preparation of the transplants followed previously reported procedures [4]. Nine transplants were separated for determination of the 0-month exposure level functioning as reference levels (RL). A total of 50 transplants (of about 1 g each) were vertically positioned in a polluted area [5], viz. the ITN campus on February 2001 in three different exposure systems (Fig. 1). The Fi system is allowing free influx, the Hi system has a cover shielding transplants from direct vertical deposition and the Vi system consists of a vertical white polyethylene tube (0.5-m diameter, 1.5 m high and 3 mm thick) placed over a metallic support to

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Fig. 1 Transplants exposure systems—From *left to right*: Vi, two Fi systems and two Hi systems

prevent any direct lateral element deposition on the lichen transplant. Both Fi and Hi systems rotate to be in line with the wind direction. Two Fi and two Hi systems were put in parallel, each having ten transplants: five facing (f) the wind (Fi_f1, Fi_f2 on Fi system and Hi_f1, Hi_f2 on Hi system) and five shielded (s) from the wind (Fi_s1, Fi_s2 on Fi system and Hi_s1, Hi_s2 on Hi system). Within the Vi set-up, two transplant sets (Vi_1 and Vi_2) were put inside the tube, with five transplants each.

Transplant sample preparation and analyses

Samples were collected on a monthly basis and cleaned by rinsing with distilled water. They were freeze-dried and ground in a Teflon ball mill for 10 min, which together with the sample had been immersed before in liquid nitrogen for 2 min. Element contents were determined by Instrumental Neutron Activation Analysis (INAA, k_0-standardisation [6]) and Particle Induced X-ray Emission (PIXE [7]). INAA analysis was carried out using pellets of 500 mg irradiated at the Portuguese Research Reactor (RPI) together with 0.1% Au–Al foil (IRMM-530R) as comparators and a high-purity germanium detector for gamma spectra determination. Concentrations were obtained by the k_0-factor method. PIXE analysis was made using a pellet of a thin layer of lichen powder in a boric acid support. Samples were irradiated in the Van de Graaff accelerator at ITN. The X-ray spectra were obtained with a Si(Li) detector and analysed by the AXIL program [8]. Concentrations were obtained by DATTPIXE program [9]. For both techniques quality control was pursued by analysing the IAEA-336 lichen material.

Results and discussion

Time relations: comparisons between Fi, Hi and Vi systems

The data was processed in terms of full time relations. For this, the three systems were compared over the total exposure period, by comparing slopes in time of the changing element concentrations in the transplants. First all elemental contents were normalised to the reference values. The systems were then compared by using $y = a*x + b$ relationships with y and x as the two compared systems in time (for instance slope between Fi–Hi means that Fi $= a*$Hi $+ b$). T-tests were applied to test both the significance of the regressions and the significance of the differences between slopes. Data was tested for normal distribution and results have shown that it is normally distributed. Table 1 show slopes (a values) obtained together with the standard errors, for all elements considered.

For $p_{regression} < 0.05$, regressions should be considered significant. The data indicate strong variability, and range from −2.15 (Sr in a Hi-Vi comparison) to 2.54 (Cr in a Fi–Hi comparison): they suggest both net accumulation and release. Table 2 gives p-values for both regression and slopes: at $p < 0.05$, regression was significant and slopes were significantly different from unit value (95% probability level). Based on significant regression and slope data only, Tables 1 and 2 suggest that Fi shows higher accumulation of Cr, Fe, La, and V than Hi; Hi shows higher accumulation of K, Pb, and Se than Fi; no differences are present between Fi and Hi systems for Ca, Sb, Ta, and Zn, that Vi releases Rb, and that Vi shows

Table 1 Results for slopes *a* (and associated standard error = SE) in comparisons between the three different hanging systems Fi (free influx), Hi (horizontal influx) and Vi (vertical influx) during the 5 months of exposure for 35 elements

	Slope Al	SE	Slope As	SE	Slope Br	SE	Slope Ca	SE	Slope Ce	SE
Fi–Hi	1.14	0.45	0.48	0.54	0.33	1.10	0.90	0.25	0.36	0.82
Fi–Vi	0.75	0.73	-0.43	1.17	−0.28	0.42	-0.46	0.34	0.05	0.31
Hi–Vi	0.42	0.56	0.42	1.12	−0.24	0.18	−0.53	0.30	0.36	0.04
	Cl		Co		Cr		Cs		Cu	
Fi–Hi	0.36	0.43	−0.09	0.66	2.54	0.39	0.85	0.48	0.23	0.31
Fi–Vi	0.56	0.39	−0.84	0.61	−0.04	0.60	0.15	0.42	0.66	0.58
Hi–Vi	−0.37	0.58	−0.45	0.63	0.03	0.23	0.47	0.24	0.95	1.06
	Fe		Hg		Hf		K		La	
Fi–Hi	1.74	0.35	0.75	0.52	0.62	0.24	0.48	0.09	2.15	0.63
Fi–Vi	−0.34	0.64	1.05	0.67	0.19	0.10	0.16	0.08	−0.23	0.34
Hi–Vi	−0.04	0.36	0.48	0.72	0.29	0.10	0.37	0.12	−0.01	0.15
	Mg		Mn		Na		Ni		P	
Fi–Hi	0.77	0.89	0.48	0.23	0.51	0.26	0.29	0.33	0.74	0.86
Fi–Vi	0.60	1.34	0.08	0.34	0.79	1.46	−0.90	0.65	−0.66	0.39
Hi–Vi	−0.67	0.71	−0.12	0.55	−0.35	2.24	0.90	1.20	0.08	0.32
	Pb		Rb		S		Sb		Sc	
Fi–Hi	0.77	0.05	0.91	0.97	1.42	0.64	1.19	0.18	1.08	0.85
Fi–Vi	0.90	0.59	−0.64	0.19	−0.62	0.82	0.41	2.88	−0.05	0.31
Hi–Vi	1.02	0.83	−0.15	0.20	0.21	0.48	1.30	2.23	0.20	0.13
	Se		Si		Sm		Sr		Ta	
Fi–Hi	0.32	0.04	0.90	0.62	1.47	0.67	0.37	0.13	1.01	0.23
Fi–Vi	−0.04	0.13	0.23	0.55	−0.20	0.41	−0.85	0.80	−0.17	0.41
Hi–Vi	−0.02	0.41	0.50	0.28	0.15	0.21	−2.15	1.76	0.00	0.39
	Th		Ti		V		Zn		Zr	
Fi–Hi	1.76	0.71	0.77	0.49	1.21	0.14	1.01	0.25	0.57	0.43
Fi–Vi	0.07	0.46	0.13	0.48	1.36	1.05	−0.57	0.92	0.14	0.09
Hi–Vi	0.24	0.16	0.10	0.42	1.17	0.82	−0.22	0.88	0.10	0.11

The notation is $y = a*x + b$, with e.g. Fi–Hi as y and x respectively

higher accumulation of Ce than Hi. Of the 35 elements and consequently the 105 possible combinations, only 13 elements show significant regressions (Table 2), indicating that the systems present comparable time-trends for that element. It should be noted also that, although the majority of the regressions are not significant, Table 1 shows negative slope values for a number of elements. Most of the negatives were related to Vi systems. For several elements, Vi may thus be interpreted as resulting in (increased) releases of element content rather than in accumulation.

Elemental contents in lichen transplants positioned differently within Fi and Hi system

The definition of the standardised difference (z-score) complies with the use of z-values in proficiency testing [10, 11], and has been applied in many studies [12–15]. The zeta-score is an estimate of the standard uncertainties of the results and shall be interpreted in the same way as the z-scores. When zeta is equal to three units of standard deviation, it means that 99.7% of all data should be within $\pm 3\sigma$ of the mean. Within Hi and Fi systems, transplants

Table 2 *p*-Values for the system comparisons in element accumulation

	p_{slope} Al	$p_{regression}$	p_{slope} As	$p_{regression}$	p_{slope} Br	$p_{regression}$	p_{slope} Ca	$p_{regression}$	p_{slope} Ce	$p_{regression}$	p_{slope} Cl	$p_{regression}$	p_{slope} Co	$p_{regression}$
Fi–Hi	0.530	0.087	0.097	0.437	0.248	0.783	0.431	**0.035**	0.158	0.691	**0.028**	0.466	**0.020**	0.895
Fi–Vi	0.481	0.380	0.052	0.736	**0.002**	0.545	**0.001**	0.276	**0.002**	0.884	0.065	0.248	**0.002**	0.260
Hi–Vi	0.083	0.506	0.310	0.733	**0.000**	0.281	**0.000**	0.177	**0.000**	**0.003**	**0.006**	0.571	**0.007**	0.529
	Cr		Cs		Cu		Fe		Hf		Hg		K	
Fi–Hi	**0.001**	**0.007**	0.519	0.176	**0.005**	0.504	**0.009**	**0.015**	**0.023**	0.080	0.346	0.249	**0.000**	**0.012**
Fi–Vi	**0.018**	0.952	**0.010**	0.747	0.261	0.340	**0.010**	0.637	**0.000**	0.139	0.867	0.214	**0.000**	0.132
Hi–Vi	**0.001**	0.916	**0.007**	0.144	0.918	0.437	**0.003**	0.910	**0.000**	0.063	0.181	0.549	**0.000**	0.055
	La		Mg		Mn		Na		Ni		P		Pb	
Fi–Hi	**0.015**	**0.042**	0.588	0.451	**0.007**	0.123	**0.013**	0.145	**0.008**	0.442	0.545	0.452	**0.000**	**0.001**
Fi–Vi	**0.001**	0.547	0.539	0.687	**0.004**	0.835	0.764	0.625	**0.003**	0.263	**0.001**	0.186	0.734	0.225
Hi–Vi	**0.000**	0.969	**0.006**	0.414	**0.010**	0.841	0.248	0.885	0.862	0.508	**0.003**	0.816	0.961	0.307
	Rb		S		Sb		Sc		Se		Si		Sm	
Fi–Hi	0.838	0.418	0.216	0.113	0.072	**0.006**	0.843	0.293	**0.000**	**0.005**	0.740	0.245	0.193	0.117
Fi–Vi	**0.000**	**0.045**	**0.011**	0.501	0.672	0.895	**0.002**	0.892	**0.000**	0.764	**0.036**	0.704	**0.003**	0.655
Hi–Vi	**0.000**	0.516	**0.021**	0.691	0.776	0.600	**0.000**	0.216	**0.005**	0.962	**0.017**	0.178	**0.001**	0.542
	Sr		Ta		Th		Ti		V		Zn		Zr	
Fi–Hi	**0.000**	0.066	0.934	**0.023**	0.075	0.090	0.353	0.212	**0.030**	**0.03**	0.959	**0.029**	0.087	0.279
Fi–Vi	**0.007**	0.367	**0.003**	0.706	**0.010**	0.893	**0.015**	0.800	0.491	0.288	**0.019**	0.580	**0.000**	0.198
Hi–Vi	**0.016**	0.309	**0.005**	0.991	**0.000**	0.244	**0.009**	0.824	0.665	0.250	**0.036**	0.817	**0.000**	0.433

Approaches are given Table 1 (see legends), for Fi (free influx), Hi (horizontal influx) and Vi (vertical influx) for 35 elements. $p_{slope} < 0.05$ indicate that slopes ≠ unit value (95% level, marked bold). $p_{regression} < 0.05$ indicate significant regressions (95% level, marked bold)

were positioned either shielded (s) from the incoming wind by their substrate, or such that they faced the wind continuously (f). The zeta-score (ζ) comparisons were made in terms of elemental accumulation [11]. Here, zeta-scores were calculated for every combination "f" and "s" as:

$$\zeta = \frac{|av_f - av_s|}{\sqrt{\sigma_f^2 + \sigma_s^2}} \tag{1}$$

with average av and standard deviation σ. When z is equal to three units of standard deviation, it means that 99.7% of all data should be within $\pm 3\sigma$ of the mean. The combinations F and S were considered as different from each other when $|z| > 3$.

The results for Fi and Hi systems, in all possible "f" and "s" combinations, are presented in Table 3. The data are given on a monthly basis, and generally suggest that "f" and "s" positioning towards wind direction do not result in significant differences in response in terms of element accumulation. Only a few significant differences were observed. Elements with zeta > 3 may here also be grouped on basis of the sign of the deviation. For $Fi_f - Fi_s$ positive differences ($Fi_f > Fi_s$) were observed for K (1st month), Br (3rd month), Ce, Cs and Th (4th month) and negative differences for Co, P, Pb, Sr, and Zr (3rd month), Hf and Sm (5th month). For $Hi\__f - Hi\__s$ positive differences were observed for Cl (1st month), Na and Br (5th month) and negative differences for Pb (1st month), Zr (3rd month). Although generally being rare, differences between wind-facing and wind-shielded transplants seem to be more frequent with Fi systems. A further observation is that sea-related elements may accumulate to higher levels in wind-shielded rather than wind-facing Hi systems.

Other studies have also used transplants of the epiphytic lichen *Parmelia sulcata* suspended in nylon bags using the Hi system device [15], 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 (sorientation) exposed in a very polluted area of Portugal. The data was 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 s-orientations. Similar results were obtained, both

Table 3 Zeta-scores for comparison in wind-directional positioning of transplants (Fi and Hi systems)

	Fi_F − Fi_S					Hi_F − Hi_S				
	1	2	3	4	5	1	2	3	4	5
Mg	−0.5	0.6	0.7	0.6	−0.6	1.5	0.0	**15.3**	−2.3	−0.3
Al	0.0	0.9	0.7	0.9	−0.5	−0.1	2.5	0.2	−0.3	−1.8
Si	0.2	0.7	0.2	1.2	−0.4	−0.4	2.5	0.9	−0.3	−0.8
P	1.9	−0.6	**−3.4**	−0.4	−0.2	−0.6	0.3	0.6	−0.9	−0.7
S	−0.5	−0.5	−0.7	0.9	−0.4	0.6	0.7	1.3	−0.3	0.5
Cl	−0.4	0.3	−0.7	−1.0	1.0	**6.2**	1.8	2.2	0.7	1.7
Ti	0.1	0.6	−1.2	1.7	0.1	−0.6	0.5	1.7	−0.2	0.1
Mn	0.5	0.7	−2.6	−0.7	−0.2	−0.6	0.0	2.4	1.1	1.3
Ni	1.5	0.4	−0.4	1.5	1.0	−2.0	1.1	1.7	0.2	0.2
Cu	0.8	0.3	−0.5	0.2	−1.8	−0.5	1.2	2.0	0.1	0.0
Sr	0.4	1.6	**−5.5**	−0.7	−0.2	−1.0	−0.3	0.9	−0.1	0.7
Zr	0.0	1.3	**−4.6**	1.3	−0.2	−1.0	2.1	**−3.8**	−1.5	−0.8
Pb	0.7	0.7	**−3.9**	−0.5	−0.6	**−3.9**	−0.1	0.8	1.4	1.1
V	1.9	0.6	1.3	1.1	−0.5	−1.0	0.8	2.5	0.2	0.0
Na	1.1	−1.0	−2.8	−1.1	−1.3	2.0	0.9	1.4	1.0	**5.3**
K	**3.0**	−0.4	−1.4	−0.1	−0.3	−1.9	0.2	0.0	−0.2	−0.3
La	0.5	1.5	0.3	2.7	−0.3	−1.5	1.6	0.6	0.6	0.5
Ca	−0.7	0.1	−0.5	0.4	−0.5	0.5	0.6	−1.7	1.2	0.7
Br	0.6	−1.0	**4.5**	0.9	−2.0	−0.2	1.2	0.7	0.9	**3.6**
As	0.4	2.7	1.2	1.6	−0.9	−0.8	1.3	−0.2	0.1	−0.2
Sm	0.1	1.4	1.1	2.0	**−7.8**	−1.1	1.8	0.1	0.3	−0.6
Sb	1.2	0.0	−0.5	0.6	0.2	−1.0	1.6	1.1	0.5	0.7
Co	0.8	0.6	**−6.2**	−0.4	−0.1	−2.1	0.4	1.7	0.0	0.0
Fe	0.8	1.1	−0.2	1.4	0.7	−1.1	1.1	0.9	0.1	0.1
Ta	1.1	−0.7	−1.3	−0.5	−0.2	−1.6	−0.5	1.2		−0.3
Sc	0.5	0.8	−0.2	1.2	1.4	−1.0	1.0	0.6	−0.1	0.0
Zn	0.8	−0.8	−1.3	0.8	−1.2	0.5	0.1	2.6	0.2	−0.4
Rb	1.2	−1.3	−1.0	−0.5	0.1	−0.1	0.5	0.6	0.8	−0.4
Cs	0.9	0.5	−0.7	3.0	−0.2	−0.7	0.7	−0.1	0.2	0.0
Hf	0.2	0.1	−0.5	0.3	**−13.7**	−1.5	0.6	0.1	−0.1	−0.7
Cr	1.7	1.4	−1.6	0.8	−0.6	−1.2	0.9	1.1	0.4	0.0
Th	0.1	0.6	−0.2	**6.2**	−1.3	−0.7	1.2	1.8	0.8	−0.1
Hg	0.0	0.5	0.3	1.2	0.3	−0.3	0.8	2.7	0.2	0.0
Se	−0.3	−0.9	0.0	−0.1	−0.6	−0.5	1.0	0.2	0.6	0.2
Ce	0.6	1.7	0.7	**3.0**	−1.1	−1.5	2.4	0.6	0.0	−0.4

Shielded from the wind is indicated by S, facing the wind is indicated by F; zeta-score with absolute values >3 indicate significant differences (marked bold)

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 s-oriented transplants generally did not result in differences in source profiles reflected, nor in differences in source contributions to element levels in the transplants.

The above results were obtained for a specific lichen, thus, the results are not necessarily representative for other lichens. In fact, similar studies have started now to be performed and different results were obtained for other lichen. A trace element deposition biomonitoring experiment with transplants of the fruticose lichen *Evernia prunastri* was developed [16], aimed at monitoring the effects of different exposure parameters (exposure orientation and direct rain) and to the elements Ti, V, Cr, Co, Cu, Zn, Rb, Cd, Sb and Pb. The accumulation trends were mainly affected by the exposure orientation and slightly less so by the protection from rain. The position of the thallus during exposure affected the accumulation levels. This position was strictly defined to ensure comparability. The use of a roof (non-metallic), allowing air to circulate, to protect the lichen from direct rain appeared desirable in this study. Could it be that accumulation trends may or not be affected by the exposure orientation, depending on the chosen lichen? More work should be developed in this area to answer the question raised.

Conclusions

The present paper recognises both system set-ups Vi, Hi and Fi, and wind-directional positioning "s" (shielded from the wind) and "f" (facing the wind). The results suggest the absence of any wind-directional effects on element accumulation within Fi (free influx) and Hi (horizontal influx) systems, but differences in response may be observed in relation with the transplant set-up systems. Here, the Vi (vertical influx) system generally shows poor results while Hi and Fi performance depends on the element involved. Overall, the present data suggest that the set-ups of transplant systems in exposure experiments are of significant relevance for the eventual results in (element-specific) transplant air pollution biomonitoring [17].

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