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Parmelia sulcata lichen transplants positioning towards wind direction (Part I): precipitation volumes, total element deposition and lichen element content

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Abstract *Parmelia sulcata* transplants were used in three different exposure systems, focused on three different influxes: free influx, horizontal influx and vertical influx. The total element deposition and the precipitation volumes were found to be positively correlated for Fe and Ni only. The element contents in lichen transplants and in total element deposition showed significant correlations for Ca, Fe and Mn in the free influx system and for Na, Ni and V in the horizontal influx system. No significant positive correlations were found for the vertical influx. The results indicate that, apart from response rates, the transplant positioning systems may have effects on element-specific net accumulation.

Keywords Lichen transplants · Wind influxes · Trace elements · Total deposition · Precipitation

Introduction

With lichen transplants, the material is generally positioned without any pre-set fixed position [1]. Positioning of the lichen is not usually taken into account as a variable of importance. However, results from a lichen transplant study on trace-element air pollution in Portugal suggested that both the positioning of the transplants towards the wind direction and the rate of precipitation were relevant factors in eventual data interpretation [2]. In later Portuguese studies, the transplants were fixed towards the wind direction, and vertically covered (see Fig. 1d) [3–7]. The studies, however, did not give any clue as to what extent these measures affected the eventual results obtained. This study comprises three transplant-positioning approaches; free, horizontal covering, and vertical covering (newly introduced transplant positioning system).

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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 (see Fig. 1a) [8]. Preparation of the transplants followed previously reported procedures [9]. 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 [10], viz. the ITN campus on February 2001 in three different exposure systems at a fixed height of 1.5 m above the soil. The transplants were exposed in three different systems: free influx (Fi), horizontal influx (Hi) and vertical influx (Vi) (Fig. 1). The Fi system allows 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 prevent any direct lateral element deposition on the lichen transplant (see Fig. 1e). 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 10 transplants (see Fig. 1c

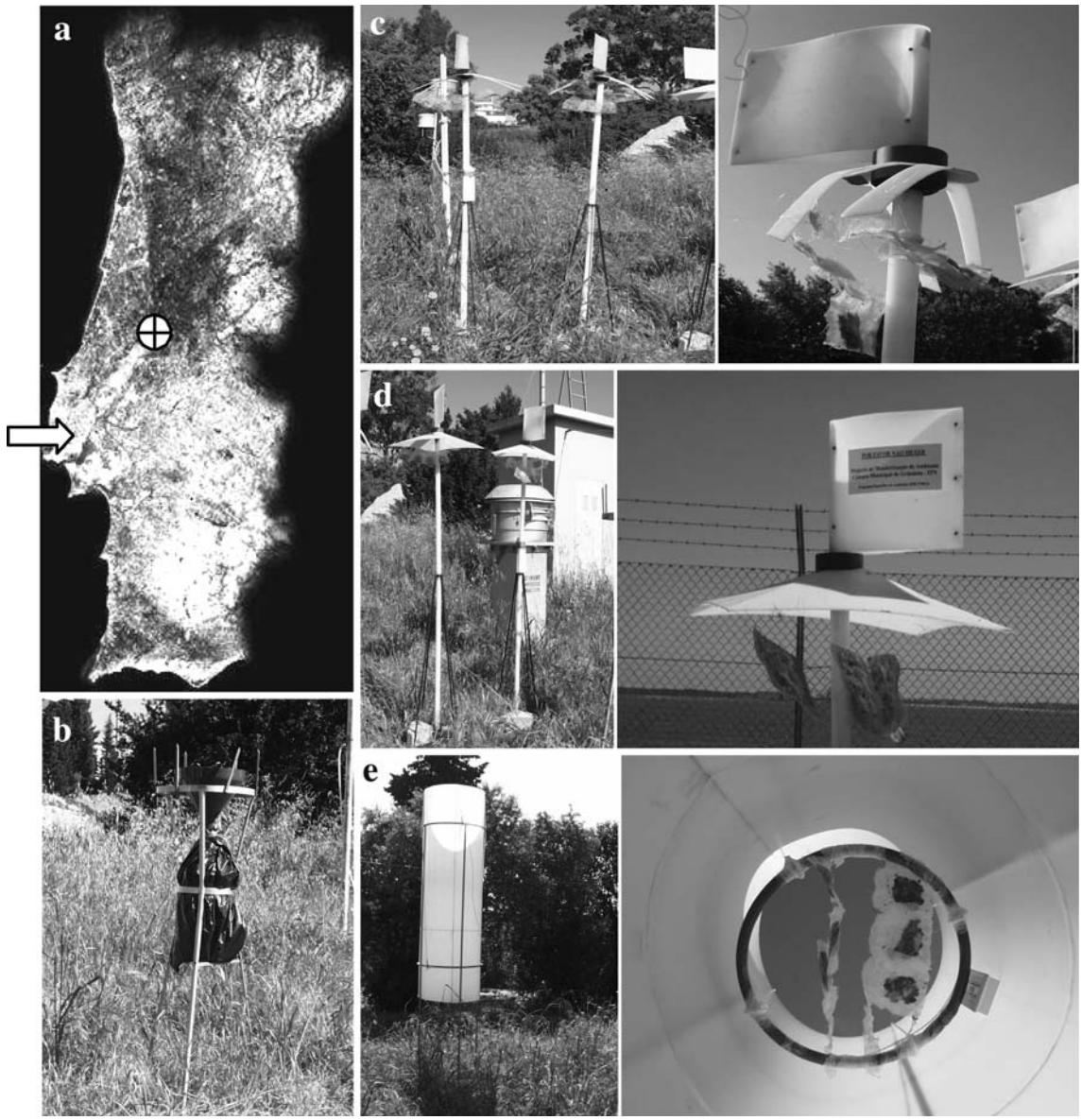

Fig. 1 Geographical map of Portugal with collection site (*circled plus* Fátima) and exposure site (*double arrow* ITN campus, Sacavém) (**a**); Water collection (**b**); Hanging systems: Fi with detail (**c**); Hi with detail (**d**); and Vi with detail (**e**)

and d respectively): 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.

Water sampling

Total element deposition was monthly collected using a 25 cm diameter funnel on top of a 10 L polyethylene bucket (see Fig. 1b). The bucket was covered with a non-transparent (black) plastic bag to avoid light interference. After collection, the water volume was determined and all samples passed through the 125 μm nylon net to retain possible small floating insects. Samples were acidified with 0.5 mL 67% HNO_3 for each 1 L of water collected, to maintain pH lower than 2, thereby avoiding formation of organic material. The samples were then frozen.

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) and Particle Induced X-ray Emission (PIXE) [11, 12]. 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. 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 [13]. Concentrations were obtained by DATTPIXE program [14].

Water sample preparation and analyses

The collected water samples were analysed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) equipped with a Perkin Elmer As-93Plus Autosampler (Perkin-Elmer, Shelton CT, USA). For concentrations below ICP-OES detection limits a Perkin Elmer Ultrasonic Nebulizer (USN 6000^{+}) was coupled. Ten measurements per wavelength were made and a flow rate of 1.50 mL min^{-1}. The analyses were performed at the Faculty of Applied Sciences – Section Radiation and Isotopes for Health (RIH), at Delft, The Netherlands.

Results and discussion

Vi set-up characteristics, relative to Fi and Hi set-ups

The Vi set-up was considered a set-up for which additional measurements were necessary to characterise this set-up relative to the Fi and Hi ones, for factors thought relevant for the general performance of the transplant lichens (temperature, light and turbulence). Overall measured temperatures were regarded as similar for the three systems. The reduced luminance inside system Vi (about 50%) was assumed as not significantly affecting lichen photosynthetic rates, relative to the other systems. Turbulence was negligible in the middle of the tube: in Vi system transplants were put at that position within the tube, and the total tube position was adjusted to assure transplant positioning at a height of 1.5 m above soil level.

Lichen element content compared to the reference level (RL)

Data was tested for normal distribution and results have shown that it is normally distributed. Student *t*-tests [15] were used to test the significance ($p < 0.05$) of differences between the lichen element data from the three hanging systems and the initial levels, serving as RL, all irrespective of wind-directional positioning. The results (Tables 1, 2, 3) indicate that transplant element contents should be considered not significantly different from RL values for the full 5-month exposure period for Cs, Hf, K, P, Rb, Se, Si and Sr (Fi system), Cr, Cs, Fe, Hf, La, P, Rb, Sc, Si, Sm, Sr, Th and Zr (Hi system), and Al, As, Cr, Cs, Fe, K, Mg, Sb, Sc, Si, Sm, Sr, Ti and Zn (Vi system). Differences from RL values were obtained for the full exposure period for Co, Mn, Na, Ni, Pb, Sb, V, and Zn (Fi system) and Mn and Na (Hi system). Time-related progressive increases in

Table 1 Probability values obtained by the application of student *t*-tests to compare lichen transplants exposed on Fi (free influx) with RL (based on nine replicates)

RL_Fi						
Al	As	Br	Ca	Ce	Cl	Co
0.059	*0.450*	*0.235*	*0.154*	*0.936*	*0.583*	0.007
0.271	0.030	*0.819*	*0.052*	0.010	0.001	0.001
0.874	0.038	0.000	*0.310*	*0.220*	0.049	0.000
0.613	0.010	*0.156*	0.001	*0.064*	*0.880*	0.000
0.050	0.008	*0.083*	0.000	*0.114*	*0.592*	0.000
Cr	Cs	Cu	Fe	Hf	Hg	K
0.165	*0.450*	*0.079*	*0.262*	*0.890*	0.003	0.704
0.001	*0.066*	0.000	0.000	*0.254*	0.000	0.438
0.000	*0.348*	0.000	0.001	*0.674*	*0.215*	0.941
0.000	*0.388*	0.000	0.000	*0.523*	*0.079*	0.895
0.000	*0.126*	0.000	0.000	*0.770*	0.000	0.676
La	Mg	Mn	Na	Ni	P	Pb
0.869	0.004	0.003	0.000	0.020	*0.518*	0.012
0.020	*0.433*	0.002	0.000	0.000	*0.095*	0.000
0.144	*0.070*	0.000	0.000	0.001	*0.066*	0.000
0.084	0.038	0.000	0.001	0.000	*0.100*	0.000
0.005	*0.709*	0.000	0.000	0.000	*0.123*	0.000
Rb	S	Sb	Sc	Se	Si	Sm
0.623	*0.262*	0.031	0.540	0.336	0.159	0.967
0.296	*0.218*	0.033	0.011	*0.191*	*0.062*	0.006
0.483	0.001	0.000	*0.076*	*0.292*	*0.783*	*0.337*
0.067	0.000	0.000	0.021	*0.086*	*0.364*	*0.063*
0.417	0.000	0.000	0.003	*0.607*	*0.057*	0.001
Sr	Ta	Th	Ti	V	Zn	Zr
0.257	*0.179*	*0.499*	*0.083*	0.011	0.025	*0.403*
0.563	0.002	0.018	0.005	0.000	0.000	*0.517*
0.125	*0.066*	*0.696*	0.001	0.000	0.002	0.026
0.193	*0.064*	*0.387*	0.002	0.000	0.000	*0.065*
0.238	0.130	0.015	0.000	0.000	0.000	*0.813*

Results higher than 0.05 are marked italicized

Table 2 Probability values obtained by the application of student *t*-tests to compare lichen transplants exposed on Hi (horizontal influx) with RL (based on nine replicates)

RL_Hi						
Al	As	Br	Ca	Ce	Cl	Co
0.264	*0.091*	*0.238*	*0.273*	*0.229*	*0.162*	0.035
0.028	0.028	0.043	0.041	*0.294*	0.000	*0.124*
0.054	*0.816*	*0.396*	*0.340*	*0.334*	0.000	*0.121*
0.473	*0.704*	0.004	*0.123*	*0.655*	0.000	0.042
0.069	*0.075*	*0.073*	*0.194*	0.043	0.000	0.010
Cr	Cs	Cu	Fe	Hf	Hg	K
0.056	*0.599*	*0.099*	*0.503*	*0.634*	0.006	*0.610*
0.064	*0.303*	*0.072*	*0.191*	*0.750*	0.010	*0.755*
0.636	*0.638*	0.035	*0.473*	*0.427*	*0.360*	*0.062*
0.385	*0.429*	0.001	*0.364*	*0.146*	0.003	0.032
0.674	*0.535*	0.001	*0.430*	*0.122*	0.016	0.039
La	Mg	Mn	Na	Ni	P	Pb
0.759	*0.395*	0.008	0.000	*0.147*	*0.264*	*0.078*
0.597	*0.556*	0.020	0.001	0.008	*0.757*	0.002
0.554	*0.698*	0.026	0.001	0.013	*0.433*	*0.146*
0.636	*0.757*	0.000	0.000	0.002	*0.342*	0.005
0.703	0.016	0.001	0.000	0.001	*0.587*	0.015
Rb	S	Sb	Sc	Se	Si	Sm
0.744	*0.093*	*0.221*	*0.585*	*0.777*	*0.617*	*0.747*
0.811	0.000	0.021	*0.344*	*0.381*	*0.177*	*0.237*
0.143	0.000	0.025	*0.657*	*0.100*	*0.123*	*0.336*
0.166	0.000	0.004	*0.983*	0.004	*0.412*	*0.936*
0.646	0.000	0.002	*0.841*	*0.830*	*0.823*	*0.391*
Sr	Ta	Th	Ti	V	Zn	Zr
0.874	*0.316*	*0.820*	*0.632*	*0.100*	*0.107*	*0.616*
0.683	0.031	*0.343*	*0.144*	0.039	0.017	*0.273*
0.370	*0.201*	*0.731*	*0.165*	*0.051*	*0.267*	*0.442*
0.107	*0.853*	*0.520*	0.035	0.007	0.018	*0.297*
0.677	*0.919*	*0.149*	*0.081*	0.013	0.035	*0.527*

Results higher than 0.05 are marked italicized

Table 3 Probability values obtained by the application of student *t*-tests to compare lichen transplants exposed on Vi (vertical influx) with RL (based on nine replicates)

RL_Vi						
Al	As	Br	Ca	Ce	Cl	Co
0.493	*0.645*	*0.178*	0.021	*0.301*	0.014	*0.157*
0.126	*0.300*	0.022	*0.071*	*0.222*	0.010	*0.068*
0.306	*0.443*	*0.375*	*0.852*	*0.545*	*0.453*	0.006
0.584	*0.318*	*0.423*	*0.332*	0.033	*0.921*	*0.604*
0.854	*0.625*	*0.231*	*0.675*	0.036	*0.957*	*0.520*
Cr	Cs	Cu	Fe	Hf	Hg	K
0.939	*0.698*	0.013	*0.560*	*0.962*	*0.111*	*0.114*
0.054	*0.380*	*0.099*	*0.073*	*0.161*	0.005	*0.112*
0.258	*0.987*	0.010	*0.432*	*0.327*	*0.873*	*0.662*
0.762	*0.118*	0.011	*0.195*	0.028	*0.183*	*0.090*
0.174	*0.193*	0.031	*0.246*	0.027	*0.856*	*0.571*
La	Mg	Mn	Na	Ni	P	Pb
0.618	*0.349*	0.009	0.000	*0.079*	0.035	*0.119*
0.364	*0.571*	0.016	0.000	0.005	*0.127*	*0.500*
0.469	*0.526*	0.001	0.000	0.004	*0.291*	0.013
0.106	*0.140*	*0.304*	0.003	*0.126*	*0.965*	0.041
0.049	*0.785*	0.006	*0.191*	0.002	*0.585*	*0.150*
Rb	S	Sb	Sc	Se	Si	Sm
0.461	*0.080*	*0.514*	*0.816*	*0.438*	*0.823*	*0.539*
0.116	0.006	*0.063*	*0.139*	*0.678*	*0.185*	*0.210*
0.012	0.006	*0.168*	*0.820*	*0.701*	*0.558*	*0.358*
0.556	*0.313*	*0.349*	*0.072*	*0.180*	*0.203*	*0.059*
0.947	0.024	*0.101*	*0.086*	0.028	*0.234*	*0.093*
Sr	Ta	Th	Ti	V	Zn	Zr
0.834	*0.172*	*0.964*	*0.824*	*0.420*	0.127	0.031
0.780	*0.402*	*0.270*	*0.087*	0.009	0.211	*0.153*
0.913	*0.380*	*0.932*	*0.233*	0.010	0.234	0.033
0.992	*0.068*	*0.069*	*0.377*	*0.071*	*0.881*	0.010
0.886	–	0.041	*0.141*	0.040	*0.051*	0.009

Results higher than 0.05 are marked italicized

significance of the differences from RL were observed for As, Cr, Cu, Fe, Ti and S (Fi system), Cl, Ni, S, Sb and Cu (Hi system), and Cu and Zr (Vi system).

For most elements, the Fi system resulted in higher rates of element accumulation with the length of the exposure period (also shown by the highest number of elements different from RL values on Fi). It should also be noted that variances between replicates were relatively high, and probably associated to intrinsic variable behaviour of biological systems, of the order of 20% (see data on *P. sulcata* from FREITAS and NOBRE [16]). Finally, the progressive increase in differences, of the results obtained on lichen transplants exposed in the different systems from RL, suggests that exposure time may be a very relevant issue in comparisons of the transplant systems.

Lichen element content, precipitation volumes and total element deposition

The amount of rain was collected as 70, 90, 8, 31 and 14 L m^{-2} respectively for the 1st, 2nd, 3rd, 4th and 5th month of exposure. In the rainwater samples the elements

As, Ce, Co, Cr, Hg, Li, Rb, Sb, Sc, Se, Sm, Th, Ti and Y were below the limits of detection. Table 4 presents correlation values (R) for Al, Ba, Ca, Cs, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Si, Sr, V and Zn as calculated for the correlation between precipitation (in L m^{-2}) and total element deposition (in mg m^{-2}). Correlation coefficients R with $p < 0.05$ meet the 95% significance criterion and should be considered correlated. Also every time a correlation coefficient and a P-value was calculated a scatter plot was generated to visually check the correlation. The data indicate significant (positive) correlations for Fe and Ni only. For Zn, SLOOF [17] also reported the absence of any significant correlation between total element deposition and precipitation volumes; she reasoned that this may be attributed to additional effects from dry deposition and washout side effects from rainfall.

Data, presented in Table 5 shows correlations between the elements concentration in *Parmelia sulcata* transplants (Fi, Hi and Vi systems) and the total element deposition. Positive and significant correlations ($p < 0.05$) were obtained for Ca, Fe and Mn (Fi system), and for Na, Ni and V (Hi system); for the Vi system no significant correlations were found. A strong inverse correlation with total element deposition was observed for K in the Hi system and Vi system, possibly denoting some membrane damage in the transplants exposed within these systems. The release of K is related to cell membrane damage, which has notable consequences for the loss of electrolytes, particularly K and Mg [18]. For the systems used, temperature may be ruled out as in causing losses in lichen vitality (increases in membrane permeability), since all systems experience similar temperatures. Future study may give additional attention to humidity: possibly the systems don't share the same humidity regimes and that can influence the biomonitor response [19]. Another noteworthy observation is that the transplants within the Vi system, (although none significant), show a number of negative correlations with element deposition (e.g. for Al, Ca, Cs, Fe, Mn, Na, Si, Sr and Zn, that is nine out of the 16 elements considered). Is it possible that these elements all result from one or two similar sources of emission with a given particle dimension, which therefore has difficulties to reach the monitor exposed in this system?

Table 4 Correlation coefficient (R) between precipitation (in L m^{-2}) and total element deposition (in mg m^{-2})

	Al	Ba	Ca	Cs
R	0.348	0.616	0.188	0.475
p	0.565	0.268	0.762	0.419
	Cu	Fe	K	Mg
R	0.006	**0.930**	0.193	0.871
p	0.993	**0.022**	0.755	0.054
	Mn	Na	Ni	Pb
R	0.834	0.867	**0.950**	0.721
p	0.079	0.057	**0.013**	0.169
	Si	Sr	V	Zn
R	−0.061	0.746	0.646	0.847
p	0.923	0.148	0.239	0.070

Correlation coefficients R with $p < 0.05$ meet the 95% significance criterion (marked bold)

Table 5 Correlation coefficient (R) between lichen element contents (in mg kg^{-1}) exposed in systems Fi, Hi and Vi and cumulative total element deposition (in mg m^{-2})

Hanging system		Al	Ca	Cs	Cu	Fe
Fi	R	0.720	**0.935**	0.544	0.502	**0.879**
	p	0.171	**0.020**	0.489	0.388	**0.050**
Hi	R	0.146	0.408	−0.451	0.814	0.194
	p	0.815	0.496	0.588	0.094	0.754
Vi	R	−0.028	−0.842	−0.702	0.379	−0.576
	p	0.964	0.073	0.612	0.526	0.309
		K	Mg	Mn	Na	Ni
Fi	R	−0.820	0.840	**0.896**	0.393	0.275
	p	0.089	0.075	**0.039**	0.513	0.657
Hi	R	**−0.888**	0.735	0.592	**0.986**	**0.887**
	p	**0.044**	0.157	0.293	**0.002**	**0.045**
Vi	R	**−0.893**	0.653	−0.307	−0.382	0.571
	p	**0.041**	0.232	0.617	0.525	0.304
		Pb	Si	Sr	V	Zn
Fi	R	0.804	0.356	0.292	0.787	0.821
	p	0.101	0.557	0.634	0.115	0.088
Hi	R	0.239	−0.480	0.554	**0.981**	0.731
	p	0.694	0.413	0.333	**0.003**	0.203
Vi	R	0.364	−0.821	−0.864	0.190	−0.061
	p	0.548	0.089	0.059	0.763	0.922

Correlations coefficients R with $p < 0.05$ meet the 95% significance criterion (marked bold)

Conclusions

The overall data suggest that transplants within Fi systems may respond the fastest relative to those within Hi and especially Vi systems [20]. Vi system usually showed lowest element concentrations and no significant correlation was found with total element deposition. For 14 out of the 35 elements, Vi systems showed no differences with

RL values at all. Comparing lichen data to element total deposition, the results suggest that the Fi system should be preferred for Ca, Fe and Mn, and the Hi system for Na, Ni and V [20]; for the Vi system no significant correlations were found. Results were obtained for specific lichen, and therefore are not necessarily representative for other lichen and so more work should be developed in this area.

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