Journal of Atmospheric Chemistry **49:** 95–110, 2004.

Biological and Temporal Variations of Trace Element Concentrations in the Moss Species *Scleropodium purum* (Hedw.) Limpr.

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(Received: 23 April 2004; accepted: 5 May 2004)

Abstract. Over a period of one year, the moss *Scleropodium purum* was sampled every two weeks in a French rural area to determine the levels of Li, Na, Al, Si, P, Ca, V, Mn, Fe, Zn, Ba, Hg, and Pb. The element distribution in the moss shoot was studied throughout the year. An apical bioconcentration was discovered for Na, P, Ca, Mn, and Zn, whereas higher levels were found in the basal fraction for Li, Al, Si, V, Fe, Ba, Hg, and Pb. A significant variation of element concentrations was observed during the sampling period. In the apical part Li, Al, Si, V, Fe, Ba, and Hg show maximum levels in the summer and minimum in the autumn. The same pattern was found with Ca and Mn in the whole plant, whereas Na showed opposite fluctuations.

Key words: atmospheric deposition, bioaccumulation, bryophyte, metal, temporal variations.

1. Introduction

Owing to their morphological and physiological properties, lack of well-developed cuticle layer or root system, mosses obtain most of their nutrients directly from the atmosphere. Moreover, mosses are highly efficient in absorbing and retaining several metals from dry and wet atmospheric deposition (for reviews see, e.g., Brown, 1984; Brown and Bates, 1990; Tyler, 1990; Markert, 1993; Zechmeister *et al*., 2003a). They have been widely used over the last three decades to monitor atmospheric trace element deposition. The moss technique has been notably employed on a wide geographical scale for estimating long term temporal and spatial trends in metal deposition (Rühling, 1994; Rühling and Steinnes, 1998). Parallel to these moss surveys, several studies have focused on the improvement of the moss method. They investigated either the optimization of the sampling and chemical analysis of the moss samples (Steinnes *et al*., 1993; Fernández *et al*., 2002a; Ayrault *et al.*, 2002), or the different sources and processes other than atmospheric deposition which may influence the concentrations of elements in mosses. Several factors

were taken into account: the influence of the plant cover (Bargagli, 1998; Økland *et al*., 1999; Ceburnis and Steinnes, 2000; Fernández and Carballeira, 2002) contamination of the soil (Bargagli *et al*., 1995; Bargagli, 1998; Genoni *et al*., 2000; Fernández and Carballeira, 2001; Fernández and Carballeira, 2002) and possible seasonal variations of the elements contained in the moss. However, while the first two were well documented, differences still show in the results on seasonal variability. At different times of the year, Thöni *et al.* (1996), Berg and Steinnes (1997), Fernández and Carballeira (2002), and Zechmeister *et al*. (2003b) found no differences in the element levels, whereas Markert and Weckert (1989) and Couto *et al.* (2003) observed significant differences in major, or trace element, concentrations over the period. The main purpose of this study is to provide new data on seasonal patterns, if any.

Nevertheless, once the moss species was chosen, the main problem was to define the moss shoot fraction used for this monitoring. In some species, it is possible to identify successive seasonal growth due to the presence of well-defined annual segments (*Hylocomium splendens* (Hedw.) Br. Eur.) or variations in the space branching zone (*Abietinella abietina* (Hedw.) Fleisch.) (Zechmeister *et al.*, 2003b). Unfortunately, such clear differences of growth are not common, and in many cases it is impossible to determine age with any accuracy. Depending on the studies, the moss segments used vary, and this can lead to differences in the results. For example, as part of the European moss survey various parts of the *Scleropodium purum* plant were used: either the green and green-brown parts (Markert *et al.*, 1996), the whole shoot (Gombert *et al.*, 2003), the two-year old apical parts (Sucharová and Suchara, 1998), the three-year old apical parts (Galsomiès *et al.*, 1999), or a fixed length (3–4 cm) (Fernández *et al.*, 2002b). The first part of this article will discuss the selection of the fraction of moss best suited for temporal monitoring. The second part will broach the subject of the possibility of seasonal patterns.

2. Materials and Methods

2.1. MOSS SAMPLING

The study was carried out in a rural area, located in a forest, in the center of France (200 km south of Paris, 47°39′N, 02°06′E). This area is an extensive Scots pine (*Pinus sylvestris* L.) stand, characterized by a permanent and abundant moss carpet of *Scleropodium purum* (Hedw.) Limpr.

Following the recommendations for moss biomonitoring (Rühling, 1994), 10 sub-areas of 1 m^2 of *Scleropodium purum* carpet were defined in a 50 × 50 m area. 30 shoots were collected in each sub-area, and they were mixed to make up a sample. Samples were collected shoot by shoot, using talcum-free plastic gloves, and transported for chemical analysis to the laboratory in acid-cleaned boxes. Samples

were collected every other week over the course of one year (October 2001–2002).

2.2. CHEMICAL ANALYSIS

All further handling of the samples took place in a clean room using acid-cleaned vessels. Following air drying at 40 °C for 24 h, moss samples were cleaned to remove adhering material (plant remains and soil particles).

In order to obtain a standard length of shoot, an apical length of 2 cm was decided upon. This value matches the minimal length of the upper green part of all shoots at the beginning of the study. The 2 cm apices of the shoots were cut with ceramic scissors. Both fractions (apical and basal parts) of the plant were weighted and ground in a contamination-free centrifugal titanium mill (Fritsch™ Pulverisette 14®).

Approximately 60 mg of ground moss sample were digested for 12–15 h in 7 mL of a mixture made of 70% nitric acid (65%; Merck™ Suprapur®) and 30% Milli-Q® water in closed Teflon bombs (Savillex™ Teflon®) at 134 °C. After digestion, extracts made up a final volume of 60 mL. Measures of Li, Na, Al, Si, P, Ca, V, Mn, Fe, Zn, Ba, Hg, and Pb (as well as other elements not discussed here) were carried out using an axial torch inductively coupled plasma atomic emission spectrometer (ICP-AES, Perkin-Elmer™ Optima 3000®) equipped with an ultrasonic nebulizer (Cetac™).

Mercury analysis were performed by atomic absorption spectrometry, cold vapor (CVAAS) with an AMA-254 automatic mercury analyzer (Altec Ltd™) without acid digestion (Cossa *et al.*, 2002).

2.3. QUALITY CONTROL PROCEDURES

The absence of a significant contamination during the digestion process of the samples was checked using Teflon bomb blanks (5 for each digestion series of 49 samples) containing only nitric acid. Quality control of the analytical process was carried out using certified reference material. National Institute of Standards and Technology (NIST) standard reference materials (1515, apple leaves and 1573a, tomato leaves) were subjected to the same digestion procedure (3 for each digestion series) to estimate the precision of the extraction protocol. The National Research Council-Conseil National de Recherches Canada (NRC-CNRC) standard (SLRS-4, Saint Laurent River Water) was also used to check the accuracy of the ICP-AES measurements.

To check the homogeneity of our samples, one ground moss sample was analyzed (digestion and measurement) five times. In the same way, in order to estimate the total uncertainty of the moss analysis, including sampling, 6 moss samples, as described in paragraph 2.1, were collected at the same time and analyzed

independently. In order to validate the sampling protocol used, the spatial variability of the studied area was also tested: a moss sample was collected in each of the ten sub-areas and analyzed.

2.4. DATA ANALYSIS

For each sample, it is possible to determine the element levels present in the whole plant from the element concentrations found in the apical and basal fractions, as well as from their weight before grinding. For statistical and graphical data treatment, all values below the detection level were not used, except for the calculation for the whole plant where they were set at half the detection limit.

Data normality was assessed by a Shapiro and Wilk test ($n < 50$). Most of the elements differed from a normal or a log-normal distribution. After deciding on the basic statistic parameters, nonparametric tests were operated using Statistica© software (StatSoft, Inc).

2.5. GROWTH MEASUREMENTS

Using the tag method described by Russell (1984) and Bates (1987), growth increments of 70 *Scleropodium* shoots were monitored over the course of more than one year (October 2001–February 2003). A colored polyester thread was tied approximately 1.5 cm from the shoot apex. The distance between this tag and the apex, as well as the length of all lateral branches above the tag were measured at regular intervals.

3. Results and Discussion

3.1. AGE OF THE APICAL FRACTION

The age of the upper 2 cm fraction was calculated using growth measurements carried out in the area. The mean growth of the main stem was 2.2 cm for 381 days (Figure 1). This value was close to the one obtained by Bates (1987) in the United Kingdom, using the same species and the same technique, which was about 10.7 mm for six months (autumn–spring period). With the same species, but with a different moss measurement technique Kilbertus (1968) found an annual growth increment of 2.2 cm in the east of France. In temperate regions, *Scleropodium* appears to have the same growth increment for the main stem.

The apical part represents approximately a one-year growth, and therefore one year of contact with atmospheric depositions. Whereas the basal part, with a length varying between 4 and 8 cm, could be 2 to 4 yr old.

However, even if the interferences in growth due to the technique employed could not be neglected, the coefficient of variation was about 73%, which meant that our standardization was an approximation.

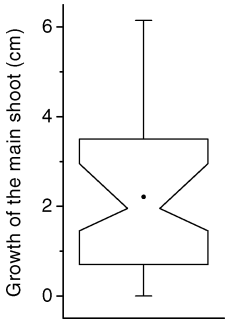

Figure 1. Notched box and whisker plot of the elongation of the main stem ($n = 42$ shoots) between January 2002 and February 2003. The upper and bottom edge of the box represent respectively the 75th and 25th percentile, the point is the mean and the neck is the median. The whiskers extend to the extreme values.

Table I. Certified values and results (mean, standard deviation (SD) and relative standard deviation (RSD)) for the analysis of NIST reference materials. a. no value given in the certificate, b. no value available. (*) $n = 6$

	SRM 1515			SRM 1573a		
$\mu g.g^{-1}$	Certified value	Mean ± SD ($n = 16$)	RSD%	Certified value	Mean ± SD ($n = 16$)	RSD%
Li	(a)	0.14 ± 0.02	12	(a)	0.53 ± 0.03	6
Na	24.4 ± 1.2	24 ± 4	17	136 ± 4	99 ± 18	18
Al	286 ± 9	288 ± 15	5	598 ± 12	528 ± 34	6
Si	(a)	428 ± 23	5	(a)	767 ± 93	12
P	1590 ± 110	1643 ± 75	5	2160 ± 40	2214 ± 106	5
Ca	15260 ± 150	12603 ± 433	3	50500 ± 900	(b)	
V	0.26 ± 0.03	0.21 ± 0.01	5	0.84 ± 0.01	0.69 ± 0.03	5
Mn	54 ± 3	47 ± 2	4	246 ± 8	207 ± 10	5
Fe	83 ± 5	66 ± 2	4	368 ± 7	300 ± 13	4
Zn	12.5 ± 0.3	11.1 ± 0.3	3	30.9 ± 0.7	26 ± 1	4
Ba	49 ± 2	44 ± 2	5	63	52 ± 3	6
Hg	0.044 ± 0.004	0.047 ± 0.003	3	0.034 ± 0.004	0.033 ± 0.001	3
Pb*	0.47 ± 0.024	0.39 ± 0.15	39	(a)	0.53 ± 0.16	29

3.2. QUALITY CONTROL

Table I summarize the data obtained for the various elements in the certified plant materials. For most elements, the relative standard deviation of reference material between the different digestion series was generally within the range 3–18%, respectively for Zn and Na. The high value (39%) observed for Pb was

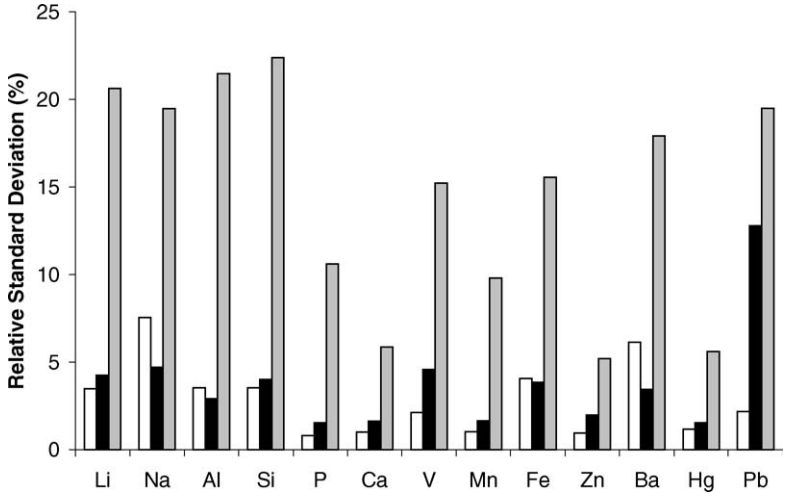

Figure 2. Relative standard deviation (RSD) obtained for the various elements analyzed in the whole moss. The white bar is for sample homogeneity ($n = 5$ samples), the black bar for sampling reproducibility ($n = 6$ samples), and gray bar for spatial variability in the studied area ($n = 10$ samples).

due to the low number of values available ($n = 6$) because of the closeness between concentration levels and the detection limit. However, moss concentration had higher values than the certified material, which could decrease this variation.

Repeated analysis of the same moss sample revealed a coefficient of variation for all the elements analyzed below 7% (Figure 2). The sampling reproducibility gave variation coefficients below 5%, except for Pb (13%). These coefficients were similar to those concerning the homogeneity of the sample. With the sampling procedure used, the variability of a moss sample is mainly due either to homogeneity of the samples after grinding, or to analytical errors.

In the 50 × 50 m study area, the spatial variability cannot be neglected. The relative standard deviation (RSD) between sub-areas can reach 23%. The fact that the variability is higher between the samples collected in the ten sub-areas than between the mixed samples, also validates the sampling protocol used.

3.3. COMPARISONS OF ELEMENTAL CONCENTRATIONS IN THE APICAL AND BASAL PARTS

Table II shows the mean and range (minimum and maximum) values of element concentrations in the moss samples throughout the year.

The values obtained with the whole plant were within the ranges reported in the 2000 French survey (Buse *et al*., 2003). For a majority of elements (except for

Table II. Element concentrations ($\mu g.g^{-1}$ dry weight) in apical or basal fractions, and in the whole *Scleropodium* shoot throughout the year; $n = 26$ samples, except Pb ($n^{**} = 20$) and Ba ($n^{*} = 25$)

	Apical fraction		Basal fraction		Whole plant		Apical /Basal	Apical /Whole
	Mean	Range	Mean	Range	Mean	Range	Ratio	Ratio
Li	0.10	0.05–0.17	0.24	0.20–0.31	0.20	0.16–0.26	0.4	0.5
Na	422	128–776	262	76–401	315	95–450	1.6	1.3
Al	161	71–310	485	379–625	376	283–507	0.3	0.4
Si	200	79–394	588	379–758	457	296–620	0.3	0.4
P	1048	837–1230	815	699–929	893	752–991	1.3	1.2
Ca	2372	1912–2868	2171	1866–2492	2241	1971–2621	1.1	1.1
V	0.56	0.29–0.90	1.43	1.20–1.71	1.14	0.92–1.40	0.4	0.5
Mn	901	670–1148	616	491–816	712	556–906	1.5	1.3
Fe	96	51–168	260	203–323	204	151–265	0.4	0.5
Zn	43	35–63	35	30–43	38	33–49	1.2	1.1
Ba	4*	2–7	8	7–13	7*	6–8	0.5	0.6
Hg	0.04	0.02–0.05	0.05	0.04–0.07	0.05	0.04–0.06	0.7	0.7
Pb	0.89**	0.50–1.43	1.76	1.38–2.34	1.42	1.01–1.83	0.5	0.6

Na and Mn), the concentrations found were below the national means. According to these results, the study area was considered as one of the less polluted sites in France.

The statistical significance of the differences between the three kinds of samples (apical, basal, whole plant) was determined by a Kruskall-Wallis test (with a 99% confidence level). Significant differences between the three fractions were found for each of the elements, except for Na and Ca. As shown in Figure 3, each fraction differed from the other two at the various sampling time. Na and Ca were the only exceptions and did not show such marked differences. A Mann-Whitney test (with a 99% confidence level) applied on the three fractions of these two elements, showed no statistical difference between the basal part and the whole plant for Na, while it showed no statistical difference between the whole plant and the apical or the basal part for Ca.

For each of the elements studied, the ratio between the apical part and the basal part, or the whole plant, was also calculated (Table II). The observed ratio between apical and basal parts divided the elements investigated into two groups. The first one (Li, Al, Si, V, Fe, Ba, Hg, Pb) showed significantly higher element concentrations in the basal part. Opposite results were obtained with the second group (Na, P, Ca, Mn, Zn). All the elements required for the biological activity of the plant were predominantly in the green fraction. Young tissues were physiologically

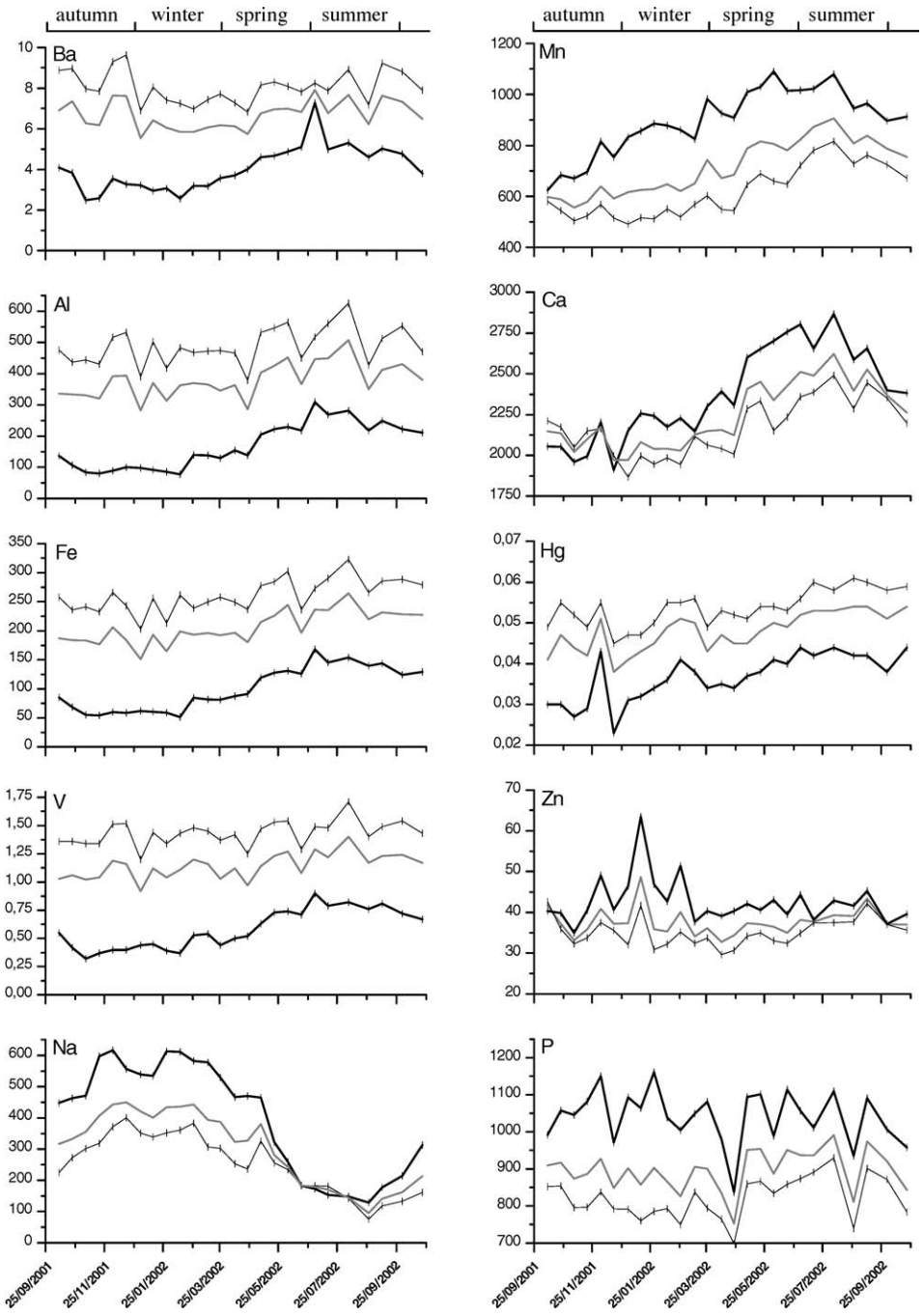

Figure 3. Element concentrations (μg.g^{-1} dry weight) in *Scleropodium* between October 2001 and 2002. The thick black line is the apical fraction, the fine black line is the basal fraction, and the gray line is the whole plant.

Table III. Results obtained from the literature and in this study on the apical-basal element distribution within moss species: Hs (*Hylocomium splendens* (Hedw.) B.S.G..), Pc (*Polytrichum commune* Hedw.), Ps (*Pleurozium schreberi* (Brid.) Mitt.), Sp (*Scleropodium purum* (Hedw.) Limpr.) and Hc (*Hypnum cupressiforme* Hedw.)

Authors	Moss species used	Apical > Basal	Apical < Basal	No difference
Tamm, 1953	Hs	P	Ca, Fe, Mn	
Rühling and Tyler, 1970	Hs		Na, Ca, Fe, Mn, Zn	Pb
Pakarinen and Rinne, 1979	Pc	Zn	Fe, Pb	Mn
Pakarinen and Rinne, 1979	Hs, Ps		Fe, Pb, Zn, Mn	
Grodzinska, 1978, Grodzinska *et al*., 1990	Hs, Ps		Fe, Pb	Zn, Mn
Bargagli *et al.*, 1995	Hs, Sp, Hc (mixed)	Na	Fe, Al, Pb, Ba, Zn, Mn	Hg, Ca
This study	Sp	Na, P, Ca, Mn, Zn	Li, Al, Si, V, Fe, Ba, Hg, Pb	

the most active part of the plant (Bates, 1979).

No figures were available specifically on *Scleropodium*, but numerous authors have reported that element concentrations in the moss shoot varied according to the age of the considered fraction (Tamm, 1953; Rühling and Tyler, 1970; Grodzinska, 1978; Bates, 1979; Pakarinen and Rinne, 1979; Grodzinska *et al*., 1990; Bargagli *et al*., 1995). Table III synthesizes the main bibliographical results.

Several explanations were given for these preferential accumulations (Brown, 1984). Enrichment in metals in the old tissues may be due to an increase of i. the number of available exchange sites further to cell degeneration, or ii. the soil influence (contamination of soil particles), but also iii. the time of exposure to the contaminants. However, depending on the studies, and most probably depending on the species used and on environmental field conditions, the distribution of macronutrients showed some variations. Internal redistribution of nutrients between moss segments, which has been reported by several authors (Brown and Bates, 1990; Brumelis and Brown, 1997; Bates and Bakken, 1998; Brumelis *et al*., 2000) may explain this distribution pattern .

Thus, depending on the length of moss shoot used in the study, this gradation of element concentrations in the plant influenced the final concentrations obtained. If using only the apical part, the concentration would be 57% lower for Fe, but 27% higher for Mn, as compared with the figures obtained with the whole plant (Table III).

3.4. MOSS ELEMENT RELATIONSHIPS

To reduce the influence of extreme values, all the concentrations were log transformed, before using a Spearman test. For apical and basal fractions, correlation coefficients calculated between the various element concentrations are shown in Table IV.

For the apical part, all the elements were significantly intercorrelated ($p < 0.01$), except for Zn and P, which show no relationships with the other elements. The highest correlations were obtained between the lithophilic elements (Li, Al, Si, V, Fe, Ba) in association with Ca and Mn. Whereas correlations involving anthropic elements (Hg and Pb) were lower. But all these elements were significantly negatively correlated with Na.

In the basal part, the element relationships were more heterogeneous. The lithophilic elements (Li, Al, Si, V, Fe, Ba) with Ca and Mn were still significantly intercorrelated, but with lower coefficients. No more significant correlation between Li, Al, Si, V, Ba, and Na were observed, even if Na was always negatively correlated with all the other elements. Moreover, Hg and Pb were not associated anymore with the crustal elements, unlike P.

3.5. TEMPORAL VARIATION

Temporal variations of several element concentrations in the moss are shown in Figure 3. For all the elements, the temporal variations observed are higher than the variability due to analytical errors. To investigate these variations, different statistical tests could be applied.

A Spearman test (with a 99% confidence level) was used to study the correlation between apical and basal fractions of the plant. Two groups could be distinguished. The first one with Na, Al, Si, Ca, V, Mn, Fe, Hg, exhibiting a significantly correlated variation between the two parts. Whereas Li, P, Zn, Ba and Pb show no significant correlation.

Even if dividing the year into seasons almost amounts to a simplification of the system (the growing seasons do not exactly coincide with the official calendar), the data can be divided into four seasons according to the sampling period and compared with each other using a Kruskal Wallis test (with a 99% confidence level). In the apical part, a significant difference was found between the four seasons for most of the elements Li, Na, Al, Si, Ca, V, Mn, Fe, Ba ($p < 0.001$) and Hg ($p < 0.01$). For P, Zn and Pb no differences were observed. In the basal part, only Na, Ca, Mn ($p < 0.001$) showed a significant seasonal variation. However, depending of the element considered, the extreme concentrations occurred in different seasons:

- In winter for the maximum values, with the minimum in summer, for Na in apical and basal parts
- In summer for the maximum values, with the minimum in winter, for Ca and Mn in apical and basal parts

Table IV. Matrices of Spearman correlation coefficients

	Li	Na	Al	Si	P	Ca	V	Mn	Fe	Zn	Ba	Hg	Pb
Apical part													
Li	1												
Na	−0.89*	1											
Al	0.94*	−0.87*	1										
Si	0.95*	−0.88*	0.98*	1									
P	−0.17	0.18	−0.10	−0.08	1								
Ca	0.78*	−0.75*	0.87*	0.84*	0.14	1							
V	0.92*	−0.87*	0.97*	0.95*	−0.09	0.85*	1						
Mn	0.71*	−0.64*	0.80*	0.76*	0.11	0.94*	0.75*	1					
Fe	0.95*	−0.89*	0.97*	0.95*	−0.12	0.87*	0.97*	0.79*	1				
Zn	−0.15	0.26	−0.10	−0.18	0.33	0.10	−0.01	0.07	−0.07	1			
Ba	0.90*	−0.87*	0.92*	0.92*	0.02	0.84*	0.89*	0.73*	0.91*	−0.08	1		
Hg	0.70*	−0.51**	0.71*	0.65*	0.08	0.75*	0.72*	0.65*	0.74*	0.19	0.61*	1	
Pb	0.59**	−0.64**	0.69*	0.58**	0.18	0.74*	0.71*	0.72*	0.73*	0.14	0.61**	0.65**	1
Basal part													
Li	1												
Na	−0.26	1											
Al	0.81*	−0.20	1										
Si	0.65*	−0.24	0.88*	1									
P	0.53**	−0.41	0.65*	0.66*	1								
Ca	0.51**	−0.79*	0.55**	0.52**	0.78*	1							
V	0.82*	−0.16	0.89*	0.75*	0.48	0.48	1						
Mn	0.64*	−0.80*	0.60*	0.54**	0.63*	0.90*	0.55**	1					
Fe	0.83*	−0.55**	0.85*	0.75*	0.54**	0.71*	0.79*	0.84*	1				
Zn	0.27	−0.34	0.45	0.51**	0.35	0.56**	0.49	0.48	0.50**	1			
Ba	0.36	−0.15	0.64*	0.63*	0.65*	0.54**	0.60*	0.32	0.46	0.63*	1		
Hg	0.52**	−0.64*	0.27	0.20	0.36	0.63*	0.45	0.75*	0.59*	0.38	0.09	1	
Pb	−0.20	−0.04	−0.15	−0.22	−0.03	0.15	0.08	−0.07	−0.12	0.38	0.37	0.08	1

Significance of correlations: * = $p < 0.001$ and ** = $0.001 < p < 0.01$.

- In summer for the maximum values, with the minimum in autumn, for Li, Al, Si, V, Fe, Ba, Hg in the apical part

According to these results, two kinds of patterns could be distinguished; the first one with Na, Ca, Mn, showing seasonal variations within the whole moss shoot (which was the fraction used), and the second one with Li, Al, Si, V, Fe, Ba, Hg, presenting significant fluctuations only in the shoot apices.

To explain these seasonal variations, four different hypotheses could be pointed out:

– Environmental parameters such as rain, which could leach through the shoot.
– Seasonal variations in the pattern of atmospheric deposition, or of deposition mode (dry, wet), which could influence the element concentrations throughout the moss.
– Dilution of the element concentrations in the shoot due to growth and biomass increase of the moss.
– Seasonal changes in the moss physiology.

Atmospheric data (temporal variation of the deposition rate and precipitation) must be taken into account to discuss which parameter may affect the results. At the present time, these data are not available, and don't allow us to select appropriate hypothesis. However, the first two hypotheses don't seem to be suitable to explain the Li, Al, Si, V, Fe, Ba, Hg variations, even if they could not be totally excluded. Indeed, they would influence the whole plant and not only the shoot apices. The most probable explanation was the dilution of the element during the autumn-winter season due to the moss biomass increase. In *Scleropodium purum,* Rincon and Grime (1989) observed the highest dry matter production in winter, which would coincide with the season of low concentration. With, Na, Ca, Mn, the variation occurred throughout the plant, and moreover concerning Na the fluctuations were similar to the growth patterns, so the same hypotheses could not be retained. Couto *et al*. (2003) obtained with *Scleropodium purum* a cyclic pattern of accumulation with Na, with maximum levels in winter and minimum levels in summer. As a possible explanation, they suggest a desiccation phenomenon with a summer alteration of the plasma membrane leading to a loss of nutrients.

Several authors have investigated the seasonal variability of element concentrations in mosses. Divergent results have been reported (Table V), mostly due to the moss species used and the study period. But the variation in atmospheric deposition in the different field sampling also has to be taken into account.

These results contradict those obtained by Fernández and Carballeira (2002) who did not find any seasonal variations with the same moss species. No explanation could be found for these differences, except for the samples being washed. Thöni *et al*. (1996), Berg and Steinnes (1997) and Zechmeister *et al*. (2003b) also came to the conclusion that there were no fluctuations. But no comparison can be made with our observations, since the sampling periods did not coincide.

Table V. Results reported in the literature on the temporal variation of element concentrations in moss. Moss species: Pf (*Polytrichum formosum* (Hedw.)), Hs (*Hylocomium splendens* (Hedw.) B.S.G.), Ps (*Pleurozium schreberi* (Brid.) Mitt.), and Hc (*Hypnum cupressiforme* Hedw.), Aa (*Abietinella abietina* (Hedw.) Fleisch.), Sp (*Scleropodium purum* (Hedw.) Limpr.)

Authors	Moss fraction and species	Sampling time	Temporal variations found
Markert and Weckert, 1989	Whole plant (Pf)	bimonthly over 2 years	Variations with Al, Fe, Pb, Ba, Ca, Zn.
Thöni *et al*., 1996	The part that was still foliated (Hs, Ps, Hc)	monthly (March till August)	No variations with Al, Fe, Pb, V, Zn.
Berg and Steinnes, 1997	Two youngest fully developed segments (Hs)	June, July and September	No variations with Li, Ca, V, Mn, Fe, Zn, Ba, Pb.
Fernández and Carballeira, 2002	3–4 cm of apical part (Sp)	monthly over 1 yr	No differences between the 4 seasons.
Couto *et al*., 2003	3–4 cm of apical part (Sp)	monthly over 3.5 yr	For Na, cyclic seasonal variations. For Al, Ca, Fe, Hg, Mn, Zn no cyclic seasonal variations.
Zechmeister *et al*., 2003b	Living parts (Aa)	monthly (July till October)	No variations for a single metal. Variations with all elements together.

On the other hand, the results obtained by Markert and Weckert (1989) and Couto *et al*. (2003) agree with the existence of seasonal concentration variations in mosses. However, in Markert, the periods of highest concentrations in Al, Ca, Fe, and Ba differ, which can be explained by the morphological differences between the two moss species used.

4. Summary

1. For each of the elements studied, *Scleropodium purum* exhibits a significant element gradient along the moss stem, representing the sampling period. Most metals (Li, Al, Si, V, Fe, Ba, Hg, Pb) are present at higher concentrations in the basal (older) shoot parts. Whereas, an apical bioconcentration was found for Na, P, Ca, Mn, Zn. This pattern has to be taken into account, when comparing results obtained with different shoot lengths.
2. A significant variation of element concentrations was observed over the sampling period. In the apical part, Li, Al, Si, V, Fe, Ba, and Hg exhibit highest levels in the summer and lowest levels in the autumn. The same pattern was found with Ca and Mn throughout the whole plant, whereas Na showed opposite fluctuations.

Therefore, moss collection has to take into account these seasonal fluctuations and cannot be carried out at any time of the year. Utmost care should apply when comparing samples collected at different seasons.

To validate these results, additional studies will have to be carried out to quantify the influence of atmospheric deposition, soil, and plant cover, on the temporal element fluctuations (Leblond *et al.*, 2003).

Acknowledgements

This thesis was financed by the French Agency for the Environment and Energy Management (ADEME). We would like to thank Daniel COSSA at IFREMER for the mercury analysis.

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