Biomonitoring of metal deposition in northern Spain by moss analysis

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Abstract

The results of the first survey carried out in northern Spain to determine atmospheric deposition of metals by analysis of terrestrial mosses, are described. Samples of different mosses, mainly *Hypnum cupressiforme* and *Scleropodium purum*, were collected from 134 sampling sites, between 1995 and 1996. Levels of Al, As, Cr, Cu, Fe, Hg, Ni, Pb and Zn, were determined by flame atomic absorption or atomic fluorescence spectrophotometry. Regression analysis was used to compare the capacity of the selected moss species to accumulate the elements, and intercalibration of accumulation in these species was carried out where necessary. Distribution maps were prepared to allow the zones most affected by metal deposition to be identified and to relate this to known sources of contamination: electricity power stations and other industries (e.g. Hg and Ni), edaphic contamination (e.g. Al and Cr) and road traffic (Pb). Background levels of metals in each species were also determined for the study area.
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1. Introduction

Terrestrial mosses have been used successfully over the past 30 years to monitor and map atmospheric deposition of contaminants in different parts of the world (e.g. Rühling and Tyler, 1970; Grodzinska et al., 1990; Sawidis and Heinrich, 1992; Himberg and Pakarinen, 1994; Thöni et al., 1996; Rühling and Steinnes, 1998; Gerdol et al., 2000). This has been possible because mosses take up nutrients, and also contaminants, directly from the atmosphere.

Compared with higher plants, mosses have several advantages which make them more suitable for this kind of study. Mosses lack a root system and a well-developed cuticle thus, on one hand

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the substrate has little influence on the levels of contaminants in their tissues and, on the other hand, they readily take up atmospheric contaminants. The cationic exchange capacity in mosses is high and they possess a high surface area to volume ratio, factors which both favour the accumulation of large amounts of pollutants.

In the 1980s, an extensive sampling programme was begun in Scandinavia on the initiative of a group of Danish and Swedish scientists. Since then sampling has been carried out every 5 years (with the financial backing of the Nordic Council of Ministers) thus providing valuable information on the patterns of deposition of contaminants in Scandinavia and adjacent regions. In 1990 the sampling programme was extended to include central and central-eastern European countries. At that time few samples were collected from southern European countries, such as Spain and Italy (Rühling, 1994).

The chief organiser of the programme, Dr Å. Rühling, invited the authors of the present study to participate in the 1995 sampling. The results presented here correspond to the part of northern Spain with the most stable climatic conditions and where the highest density of sampling sites (s.s.) was attained. Although the technique is widely known, it had never been used in many parts of northern Spain, the only data existing being from Galicia, in the extreme northwest of the country (Fernández et al., 2000b).

The results of the sampling survey carried out in 1995 and 1996 with the aim of producing the first assessment of heavy metal deposition, by moss analysis, in Spain, are presented. The main objectives of the survey were as follows:

1. To establish a network of sampling that would allow monitoring in northern Spain, following the methods of the European programme of biomonitoring using moss.
2. To determine which species of moss to use.
3. To make comparisons between species as a reference for future studies.
4. To establish patterns of distribution of the elements analysed and to identify sources of contamination and contaminated areas.
5. To make available data that can be compared with results of further surveys.

2. Materials and methods

2.1. Sampling and chemical analysis

The suggestions of Rühling (1994) were followed before choosing the species of moss to use, and these were: *Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) B., S. & G., *Hypnum cupressiforme* Hedw., *Scleropodium purum* (Hedw.) Limpr. and *Thuidium tamariscinum* (Hedw.) B., S. & G. However, during sampling we found 9 specimens of *P. schreberi* (6.8% of all the samples collected), 6 of *H. splendens* (4.5%), 103 of *H. cupressiforme* (77.4%), 68 of *S. purum* (51.1%) and 5 of *T. tamariscinum* (3.8%). Only the samples of *H. cupressiforme* and *S. purum* were analysed because the other species were less frequently found at the s.s. in the area studied (the most extended species was present at <7% of the sites).

Sampling was carried out in 1995 (April–July) and 1996 (March–July); 134 s.s. were spread throughout several provinces in northern Spain, with high densities in the eastern and western regions (Fig. 1). The number of s.s. in the whole area studied (approximately 150 000 km^2) was equivalent to a density of 0.9 sites/1000 km^2.

Collection of moss samples was performed according to the protocol adopted within the European Heavy Metal Survey (Rühling, 1994). All s.s. were located at least 300 m from highways and main roads and at least 100 m from any roads or houses. At each site, 5–10 samples were collected within a 50×50 m^2 area and combined in a composite sample. Where possible, the samples were taken from open areas and small forest clearings, located as far as possible from the nearest tree, although at some sites samples were collected under tree canopies.

In the laboratory, the apices of the shoots (3–4 cm) were cut from the moss and washed with bidistilled water. The selected material was then dried to constant weight in a forced-air oven at 50 °C. For analysis, 650 mg of homogenized moss tissue were digested with 10 ml of nitric acid (65%; Merck®, analytical grade) in a conventional microwave oven. The contents of Al, Cr, Cu, Fe, Ni, Pb and Zn in the extracts were determined using flame absorption spectrophotometry and
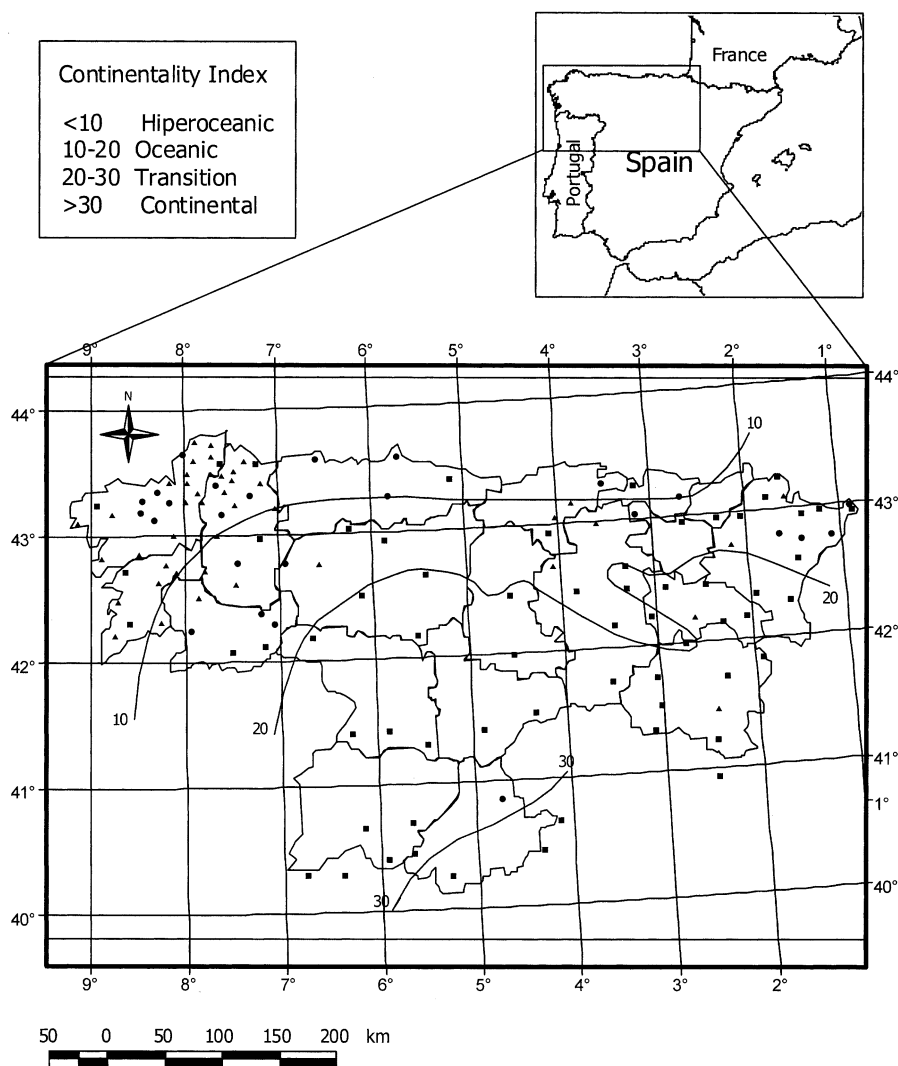
Fig. 1. Location of s.s. in Spain (■ *H. cupressiforme*, ● *S. purum*, ▲ sites where both species occurred and were collected at the same time). The map shows the oceanic-continent gradient of the study area.

those of As and Hg by atomic fluorescence spectrophotometry.

Quality control was carried out by parallel analysis of certified reference material (BCR 61, aquatic moss). The possibility of contamination during the digestion process was controlled by use of procedural blanks (1 every 10 samples). In order to minimize variations in sampling, extraction and analysis, a total of 10 replicates from one sample were analysed; the coefficients of variation

ranged between 0.2 and 12%, depending on the element.

2.2. Statistical analysis

2.2.1. Comparison between species

The differences in bioconcentration in *H. cupressiforme* and *S. purum* were compared by means of regression analysis. The metal concentrations in the tissues of two species at a s.s. are not

independent because they are related to the location of the site and the amount of metal deposition. ANOVA or other simple tests for comparison of means are therefore not applicable. In regression analysis, if the null hypothesis is true, when the pairs of values are plotted, a straight line with slope equal to 1 should be obtained. To find if there are differences between the species, it must be found whether the slope of the regression line that relates bioconcentration in *H. cupressiforme* and *S. purum* is significantly different from a slope equal to 1. We compared the slopes using the method suggested by Zar (1984).

In this study, the concentration values of one of the variables were not able to be selected, the values obtained in the field were used instead, i.e. the 'independent variable' was not really independent. In this situation, Model II regression is recommended for use instead of the usual Model I (Sokal and Rohlf, 1995; Zar, 1984); the regression was calculated as major axis regression.

2.2.2. Background levels and contamination factors

The background level of a determined element is the value representing the usual conditions of the area under study which, although affected by human activity, is well conserved (Carballeira et al., 2002). In the latter study, the authors have shown modal analysis to be a good method for estimating background levels in terrestrial mosses, and this method was therefore chosen for use in the present study. Modal analysis allows subpopulations within the element concentrations data to be identified. The subpopulation corresponding to the lowest mean concentration is assumed to consist of data from unpolluted sites (for further details, see Carballeira et al., 2002).

Once the concentration of a certain element in a moss sample is known, the following step consists of determining whether this concentration indicates contamination, i.e. if the concentration of the element is relatively high compared with the usual levels of this element. The simplest method of doing this is to calculate contamination factors (CF) for each element, i.e. the relationship between the concentration in the moss samples and the corresponding background level.

Following calculation of the CF it is possible to classify each s.s. according to the levels of each element, using the scale proposed by Fernández and Carballeira (2001). This scale, which was constructed taking into account how contaminants are dispersed in the atmosphere, allows determination of whether or not a site is contaminated, and if so the degree of contamination. The scale comprises of six categories, defined as follows: Category 1 ($CF \leqslant 1$) no contamination; Category 2 ($1 < CF \leqslant 2$) suspected contamination; Category 3 ($2 < CF \leqslant 3.5$) slight contamination; Category 4 ($3.5 < CF \leqslant 8$) moderate contamination; Category 5 ($8 < CF \leqslant 27$) serious contamination, and Category 6 ($CF > 27$) extremely serious contamination.

3. Results and discussion

3.1. Species distribution

There were notable differences in the distribution of *H. cupressiforme* and *S. purum* in the area studied. *H. cupressiforme* was widely distributed throughout the whole zone, in coastal as well as inland areas, whereas *S. purum* appeared almost exclusively in coastal areas and occurred only rarely at inland sites. According to data from the Spanish National Institute of Meteorology, the distribution zone of *S. purum* corresponds to a part of Spain with a humid temperate climate and warm summers (Anonymous, 1992). According to Gorczynski's Continentality Index (Carballeira et al., 1983), this zone is also classified as an oceanic or hiperoceanic region (Fig. 1). However, although the climate of the inland zone is also classified as temperate, it is further characterized by dry, and warm or hot summers, typical of the transition zone between oceanic and continental areas (Fig. 1). In the coastal zone the annual average rainfall is greater than 800 mm (in some mountainous regions reaching up to 2000 mm year^{-1}), whereas in the inland area, where *S. purum* was scarce, the average annual rainfall is approximately 400 mm.

Therefore, in future studies of this kind, it is recommended *H. cupressiforme* to be sampled as it is the most cosmopolitan of all the mosses studied. In areas where *H. cupressiforme* does not appear, *S. purum* should be sampled, although this

would appear only in coastal areas, where this species is widely distributed. For sampling in drier areas of Spain (S–SE) another alternative species to *H. cupressiforme* would have to be found.

3.2. Comparison between species

The results of the analyses of the moss samples are shown in Table 1. Mean and maximum levels of all metals, except Hg and Zn, were higher in *H. cupressiforme* than in *S. purum*. The coefficient of variation was similar for almost all elements in both species although the value was much higher for As and Pb in *H. cupressiforme*.

For each of the elements studied, we calculated the ratio between the concentration in *H. cupressiforme* and in *S. purum* for comparison with the corresponding ratios obtained in other similar studies (Fernández et al., 2000b; Galsomiès et al., 1999; Bargagli et al., 1995; Wolterbeek et al., 1995) (Table 2). In four out of the five studies compared, the concentrations of almost all of the elements were higher in *H. cupressiforme* than in *S. purum*.

Regression analysis was used to compare the results obtained for the two species; the regression lines corresponding to elements analysed in samples collected from sites where both species occurred, are shown in Fig. 2. The regression lines obtained for all elements were significant at $P <$

Table 2

Values of the ratio of the concentration of a given element in each of the two moss species studied (*H. cupressiforme*/*S. purum*), calculated for the present study and four other similar studies

	A	B	C	D	E
Al	1.93	1.36	1.33		
As	1.50	1.10		0.67	
Cr	1.35	1.21	1.04	0.53	1.00
Cu	1.07	1.01	0.98		1.14
Fe	1.58	1.17	1.70	0.46	1.27
Hg	0.90	1.06	1.22	0.67	
Ni	1.37	1.71	1.13		1.23
Pb	1.61	1.65	1.50		0.90
Zn	0.88	0.94	0.87	1.06	1.12

(A) This study; (B) Fernández et al. (2000b); (C) Bargagli et al. (1995); (D) Galsomiès et al. (1999); (E) Wolterbeek et al. (1995).

0.001, except for those corresponding to Hg ($P < 0.05$) and Zn ($P < 0.01$). The dashed lines represents the lines of slope equal to 1, which correspond to the hypothetical regression lines that would be obtained if there were no differences in bioconcentration in *H. cupressiforme* and *S. purum*. Comparison of the slopes of the regression lines obtained with the hypothetical lines showed that there were statistically significant differences for Al, Ni and Pb ($P < 0.01$) and for Fe ($P < 0.05$). The two species therefore must take up these

Table 1

Mean, standard deviation (S.D.), coefficient of variation (CV), minimum (Min), maximum (Max) and median concentrations of different elements ($\mu g\ g^{-1}$ d.w.) in *H. cupressiforme* and *S. purum* samples collected in northern Spain

|		Al	As	Cr	Cu	Fe	Hg	Ni	Pb	Zn
H. cupressiforme										
	Mean	1048.3	0.405	2.68	6.86	868.2	0.038	2.79	9.35	48.8
	S.D.	987.8	0.533	2.06	3.17	901.8	0.026	1.92	15.18	23.5
	CV (%)	94	131	77	46	104	70	69	162	48
	Min	243.2	0.019	0.10	2.49	138.0	0.001	0.57	1.04	8.7
	Max	5958.8	3.695	11.01	29.06	7016.7	0.177	14.10	153.0	111.3
	Median	717.3	0.204	2.51	5.98	617.0	0.037	2.28	6.59	44.6
S. purum										
	Mean	544.2	0.27	1.984	6.401	548.2	0.042	2.04	5.795	55.19
	S.D.	448	0.27	1.67	2.27	569.5	0.031	1.35	6.014	18.44
	CV (%)	82.34	99.9	84.18	35.47	103.9	73.17	66.5	103.8	33.42
	Min	156.5	0.01	0.176	3.201	135.1	0.004	0.54	0.035	25.54
	Max	2717	1.28	9.801	14.4	4029	0.203	7.76	39.15	117.7
	Median	426.3	0.19	1.682	6.021	405.4	0.034	1.77	4.262	53.69
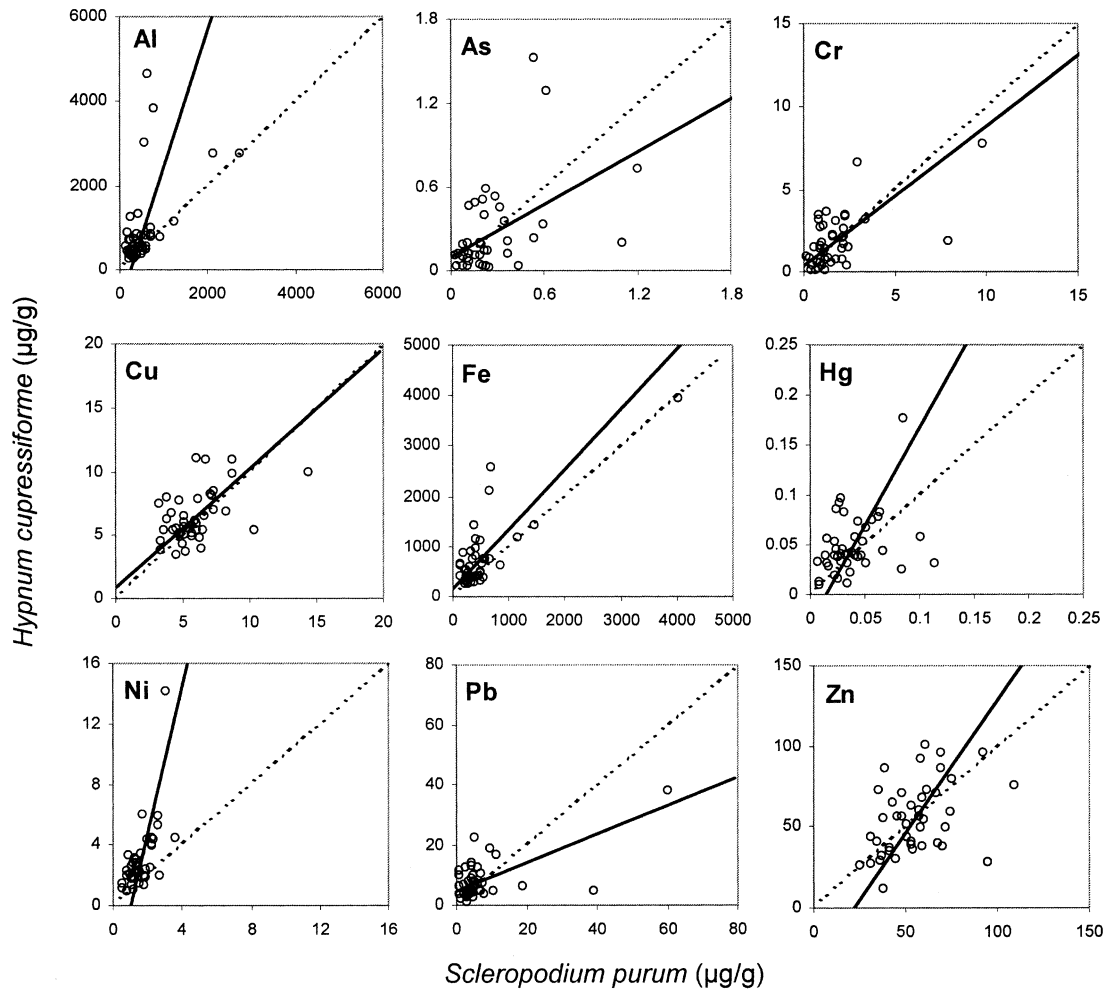
Fig. 2. Plots showing results of the interspecies comparison between *H. cupressiforme* and *S. purum*. The black line is the regression line and the dashed line is the line of slope equal to 1 (see text for details).

elements in different ways, whereas uptake of As, Cu, Cr, Hg and Zn is similar in the two species.

Taking into account the distribution of the two species studied, and given that the most of the *S. purum* samples were found in the coastal zone, the regression analysis was repeated, including only data for coastal sites at which both species were found. The results were identical to those of the previous analysis.

The interspecific differences in bioconcentration of the elements studied can be explained in various ways. Folkeson (1979) states that differences in

the way that different species accumulate contaminants are related to morphology. *H. cupressiforme* has a more branched structure than *S. purum*, and therefore a larger surface area exposed to the air, for the same weight of tissue. This clearly affects the uptake of elements that are usually present in particulate form in the atmosphere (Halleraker et al., 1998), and elements of typically mineral origin, such as Fe and Al, are better retained by *H. cupressiforme*, as shown in Table 1. Another explanation for the differences in bioconcentration is the types of mats that the two species form as

they grow. As previously pointed out by Sucharová and Suchara (1998), *H. cupressiforme* forms more dense mats than *S. purum* and is therefore able to capture elements from the atmosphere more efficiently. The third possible reason for the differences observed is the difference in growth rates of the two species, whereby differences in tissue levels of metals are brought about by a diluting effect. Zechmeister (1995) found that, compared with other species such as *P. schreberi* (which is very similar to *S. purum*), the growth rate of *H. cupressiforme* was slow, therefore even though the same amounts of contaminants were taken up by each species, there would be less dilution in the tissues of the latter and the overall concentrations of the elements would be greater. The differences in bioconcentration can therefore be explained by: different morphology, the types of mats formed and different growth rates.

3.3. Intercalibration

To avoid s.s. being selected only the basis of the presence or absence of a particular species, more than one species must be used and therefore intercalibration of the responses of the different species to varying levels of contaminants is required (Wolterbeek and Bode, 1995). This type of calibration has previously been used in similar studies carried out at both regional (Fernández et al., 2000b) and national level (Galsomiès et al., 1999; Berg and Steinnes, 1997; Thöni et al., 1996), although criticism has also been made of the intercalibration method (Wolterbeek et al., 1995; Reimann et al., 2001).

Intercalibration is carried out using the data from the s.s. where both of the selected species grow. The results can therefore be expressed in terms of a single species, in this case *H. cupressiforme*, the most widely distributed of the two species. We used the *S. purum* data as the independent variable and the *H. cupressiforme* data as the dependent variable. The intercalibration lines for those elements for which there were significant differences in bioconcentration in the two species are shown in Fig. 3.

It should be pointed out that interspecies calibration is affected by the range of concentration of elements as well as the environmental conditions of the different areas, and its use must be carefully considered and perhaps limited to correcting data in large scale studies and to mapping element distributions.

3.4. Spatial distribution of pollutants

In order to examine the dispersion of the elements studied, distribution maps of each were prepared (Fig. 4). Where possible, the data corresponding to *H. cupressiforme* were used, but where this species was not found, *S. purum* data were used and corrected using the intercalibration equations.

3.4.1. Aluminium
The regional distribution of Al in the moss samples was typical of an element available to moss almost exclusively from the soil (as suspended particles). This was clear because the distribution of the sites with the highest concentrations of Al in moss was unrelated to e.g. industrial and/or urban foci. The distribution of these points coincided, in many cases, with areas in which the lithology is dominated by schists or sandstones, which usually contain high levels of this element (Salomons and Förstner, 1984).

3.4.2. Arsenic
In the western part of the study area, high concentrations of As were associated, in the north, with the presence of two coal-fired power plants, and in the south with use of pesticides in agricultural activities (Fernández et al., 2000b). Towards the centre and the east of the zone, the highest levels of As coincided with industrial areas.

3.4.3. Chromium
Although the presence of Cr in moss was mainly due to contamination of edaphic origin, the distribution of sites with the highest levels of this element coincided with the presence of industrial foci. In the W, the levels of Cr may have been related to the presence of power plants, whereas in the E they were associated with other types of industrial installations (e.g. iron and steel mills). In the central area and in the S, the concentrations
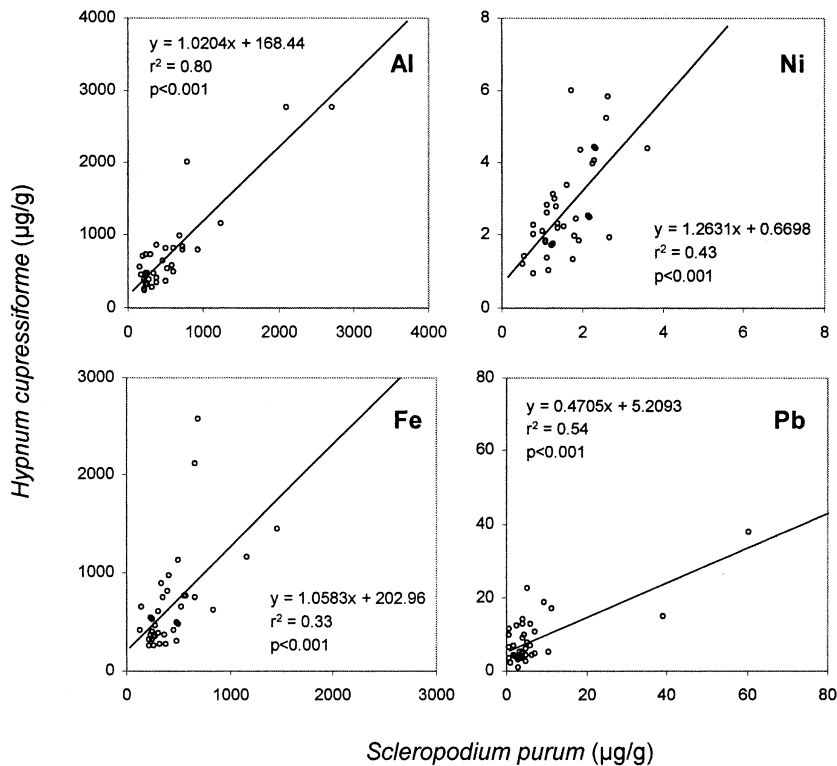
Fig. 3. Comparison of the concentrations of some of the elements determined in the mosses *H. cupressiforme* and *S. purum*.

of Cr recorded in the mosses appeared to be related to the dominant lithology of the area.

3.4.4. Copper

The pattern of distribution of this element was fairly uniform throughout the whole of the area studied. Copper can be emitted from a wide range of sources, e.g. the metal industry, mining, coal-fired plants, traffic and even soil (Rühling and Steinnes, 1998), which explains the distribution observed.

3.4.5. Iron

Uptake of this element in terrestrial mosses is mainly related to edaphic contamination, however this source alone does not account for the distribution observed in the area. Coal-fired power plant emissions also contain Fe, which explains the high levels recorded at specific points in the NW. Similarly, the presence of iron smelters in the E

accounts for the Fe levels in this area. Finally, in the central and southern zones, where erosion occurs (due to the dryness and the lack of vegetation), the pattern of distribution may be related to the presence of particles originating from the soil.

3.4.6. Lead

The levels of lead varied little throughout the area. This may be because one of the main sources of Pb is road traffic, which was present to a similar extent throughout the whole area. There are specific industrial foci, such as a coal-fired power plant in the NW and other kinds of factories in the E. The highest level of Pb ($153 \ \mu g \ g^{-1}$), recorded in the central zone, deserves special comment. The s.s. where this high level was recorded is situated in the surroundings of a factory in which Pb is recovered from used car batteries, indicating that there were serious faults in the
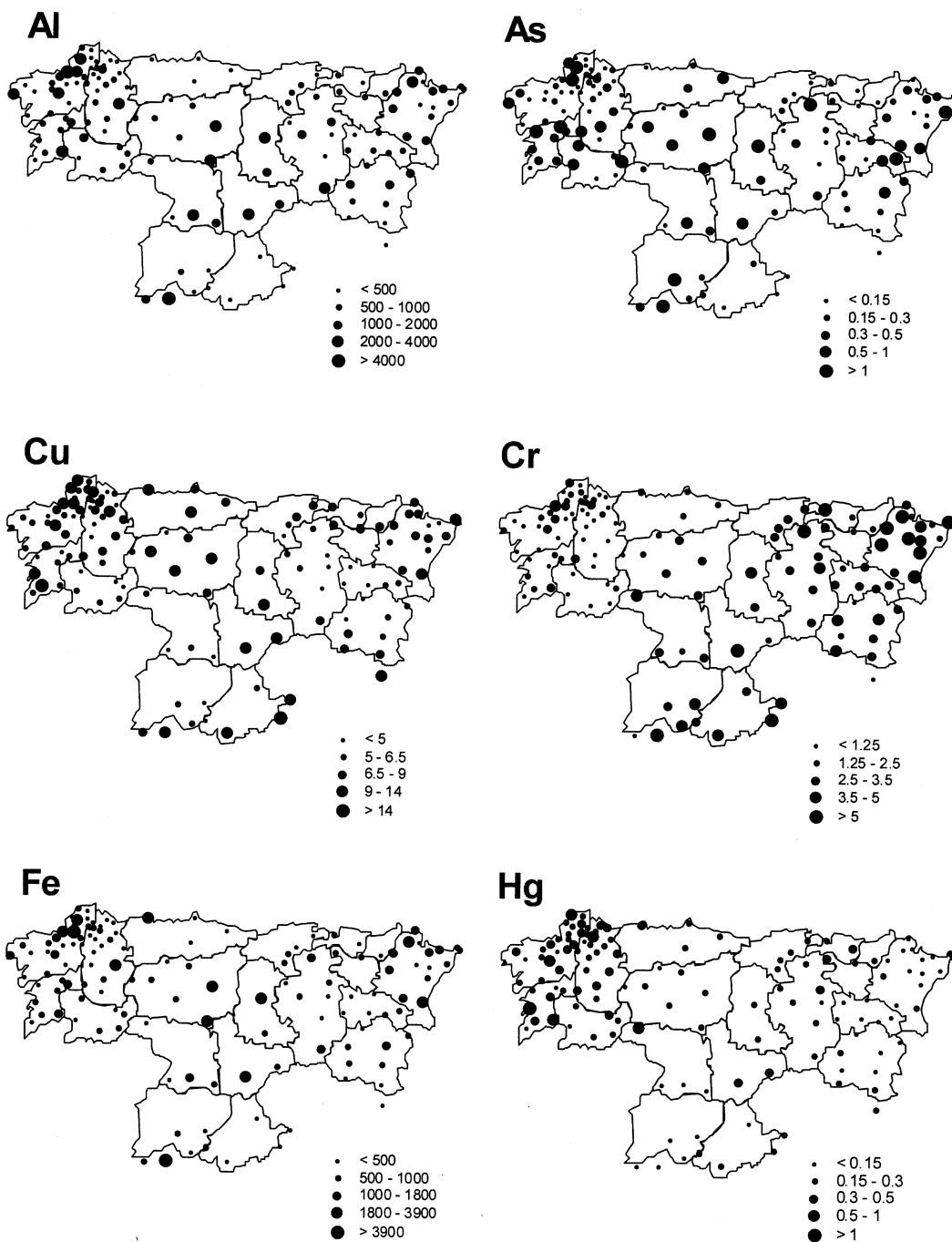
Fig. 4. Spatial distribution of elements determined (μg g^{-1} d.w.) in moss samples in the northern Spain survey. The radius of each circle is proportional to the concentration of each element.
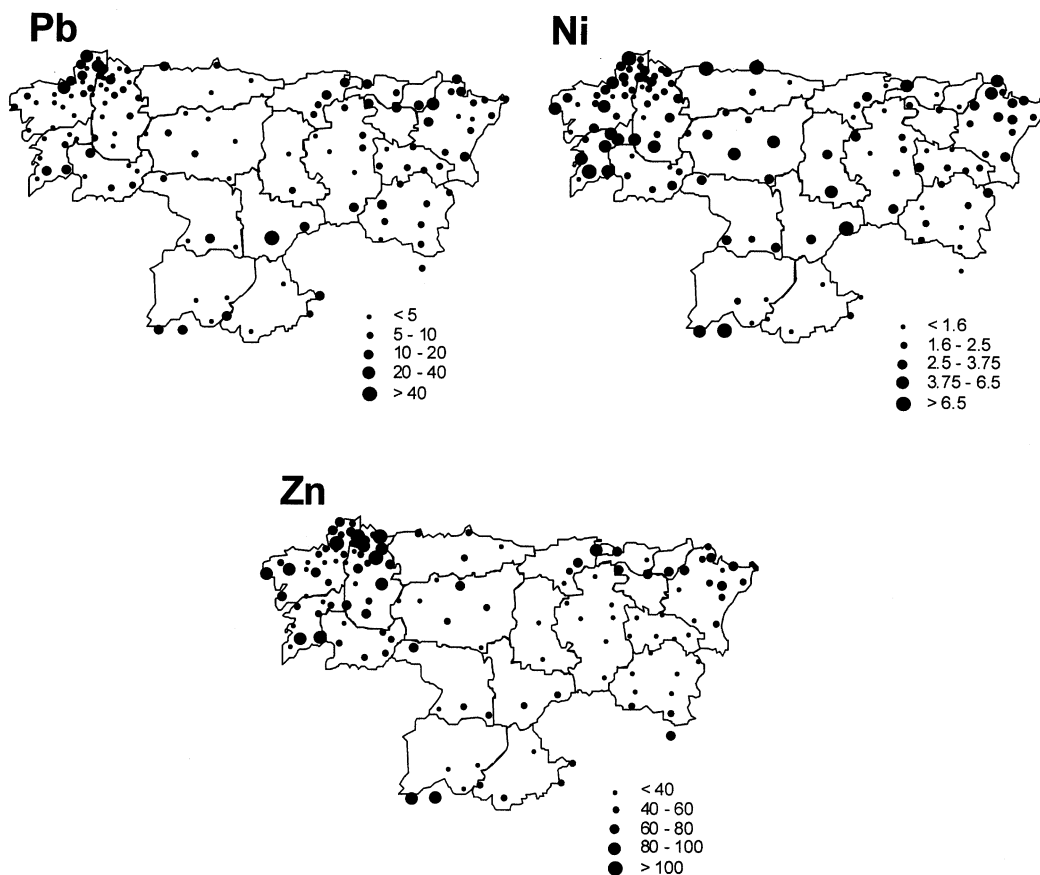
Fig. 4. (Continued).

industrial process whereby large amounts of this element were allowed to be emitted to the atmosphere.

3.4.7. Mercury

The pattern of distribution of Hg was very uniform throughout most of the area, except the NW, where there are two main sources of this element. Firstly, in the N, there are two power plants responsible for the relatively high emissions of Hg within this area (Spanish Ministry of the Environment, pers. comm.). Secondly, in the S, there is a chlor-alkali plant, around which the highest levels of Hg of the whole study area were found (Fernández et al., 2000a).

3.4.8. Nickel

The distribution of Ni was similar to that of Cu. The highest levels coincided with areas close to

different types of industrial premises: chemical factories and metallurgical, petrochemical or power plants.

3.4.9. Zinc

As with Pb, the distribution of Zn was fairly uniform throughout the whole study area, with areas of high deposition in the NW, within the range of influence of two power plants, and in the E, in the area surrounding metallurgical plants. One factor contributing to the uniformity of the levels of this element is that levels of Zn in moss are often high; furthermore, the capacity of retention of this element by mosses is relatively low, therefore, very high environmental concentrations would be required to produce high uptake of Zn (Rühling and Steinnes, 1998).

We calculated the surface area of the study zone

Table 3
Ranges of concentrations of different elements in mosses, the percentage number of s.s. at which each range occurred and the corresponding surface area

Element	Concentration range (μg g^{-1} d.w.)	Percentage of s.s. in the zone	Area (km^2)	Element	Concentration range (μg g^{-1} d.w.)	Percentage of s.s. in the zone	Area (km^2)
Al	<500	37	55 172	As	<0.15	35	52 874
	500–1000	36	54 023		0.15–0.30	26	39 080
	1000–2000	15	22 989		0.30–0.50	16	24 138
	2000–4000	10	14 943		0.50–1.0	13	19 540
	>4000	2	2299		>1.0	9	13 793
Cr	<1.25	28	42 529	Cu	<5.0	22	32 184
	1.25–2.5	25	37 931		5.0–6.5	32	48 276
	2.5–3.5	28	41 379		6.5–9.0	28	41 379
	3.5–5.0	10	14 943		9.0–14.0	17	25 287
	>5.0	8	12 644		>14.0	2	2299
Fe	<500	44	65 517	Hg	<0.025	32	48 276
	500–1000	35	52 874		0.025–0.05	38	56 322
	1000–1800	12	17 241		0.05–0.08	21	31 034
	1800–3900	8	11 494		0.08–0.10	8	11 494
	>3900	2	2299		>0.10	2	2299
Ni	<1.6	22	32 184	Pb	<5.0	42	62 069
	1.6–2.5	38	56 322		5.0–10.0	35	51 724
	2.5–3.8	23	34 483		10.0–20.0	21	31 034
	3.8–6.5	13	19 540		20.0–40.0	2	3449
	>6.5	5	6897		>40.0	1	1149
Zn	<40.0	37	55 172				
	40.0–60.0	35	51 724				
	60.0–80.0	17	25 287				
	80.0–100.0	8	12 644				
	>100.0	3	4598				

corresponding to each range of concentrations used in preparing the distribution maps (Table 3). For this, we took into account the density of s.s., the number of s.s. corresponding to each range and the total surface area studied (approximately. 150 000 km^2). For all of the elements studied, most of the surface area corresponded to the two lowest concentration ranges: between 53% of the s.s for Cr and 79% for Fe, which demonstrates that the area was relatively unaffected by deposition of the elements studied.

3.5. Background levels and contamination factors

3.5.1. Estimation of background levels
The background levels of the 9 elements studied in *H. cupressiforme* and *S. purum* (Table 4) were obtained by modal analysis.

When it is considered that the two species differ in their capacity to accumulate elements, as mentioned in previous sections, it is easy to understand why background levels must be calculated for

Table 4
Background levels (μg g^{-1} d.w.) of the elements studied, in samples of *H. cupressiforme* and *S. purum* from northern Spain

	H. cupressiforme	*S. purum*
Al	518.5	320.5
As	0.144	0.145
Cr	1.52	1.19
Cu	4.82	4.95
Fe	458.8	323.0
Hg	0.016	0.018
Ni	1.83	1.23
Pb	4.51	2.76
Zn	29.2	40.8

Table 5
Percentage distribution of s.s. in each category of contamination, according to the CF calculated from the levels of each of the elements analysed in samples of *H. cupressiforme* and *S. purum* collected in northern Spain

Category	Contamination	Al	As	Cr	Cu	Fe	Hg	Ni	Pb	Zn
1	None	30	36	30	18	32	24	25	25	22
2	Suspected	42	25	33	66	39	30	53	46	46
3	Slight	15	17	27	14	16	35	15	16	26
4	Moderate	12	12	8	1	10	10	5	10	5
5	Severe	2	8			2	1		2	
6	Extreme								1	

each. Furthermore, a particular species may not grow at some s.s. and therefore the background level in the alternative species must be known.

The data shown in Table 4 can be grouped according to whether the elements are present at higher concentrations in one or other species. The levels of Al, Cr, Fe, Ni and Pb were higher in *H. cupressiforme* than in *S. purum*, whereas the opposite was true for Zn; the levels of As, Cu and Hg were similar in both species. The differences in levels of Al, Cr, Fe, Ni, Pb and Zn were statistically significant ($P < 0.001$).

The background levels of most elements were significantly different in each moss species, therefore, although this was not true for As, Cu and Hg, it is recommended that the background level corresponding to each species is always used.

3.5.2. Contamination factors

Once the background levels are known, it is possible to calculate the value of the CF for each sample and each element. The results can therefore be interpreted, not in absolute terms, but in relative terms—on the basis of the ratio between the concentration of an element and its corresponding background level.

The values of the percentage of s.s. corresponding to each category of contamination, based on the CF values, are shown in Table 5. Only a very low percentage of the s.s. were classified in the categories corresponding to the highest degrees of contamination (categories 5 and 6). It was therefore concluded that, in general terms the mosses in the area studied were not seriously contaminated by the elements analysed.

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