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MONITORING OF AIRBORNE METAL POLLUTION BY MOSS BAGS: A METHODOLOGICAL STUDY

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Abstract: The use of moss transplants for monitoring heavy metals deposition is briefly reviewed. The methodological study concerns the effects of different types of pre-treatment on data variability. Epiphytic samples of *Hypnum cupressiforme* were collected from an unpolluted area, treated in different ways, and the resulting bags were exposed in two sites in the province of Trieste (NE Italy) with widely different pollution: a natural woodland far from urban and industrial centers, and a site near an iron smelting industry in the industrial area of Trieste. The content of eight heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb) was measured in 80 moss samples by atomic absorption spectrophotometry. The results, which represent a contribution to the standardization of the moss bags technique, concern: a) variability of metal content in mosses from natural areas: this is influenced by soil contamination; it is advisable to collect epiphytic samples, avoiding those growing near the base of the trunks; b) effects of different washing treatments on metal contents: compared to distilled water, washing with an acid solution is particularly efficient in removing metal ions associated with the cell wall, but produces more variable data; c) uptake capacity of exposed moss bags: this is not influenced by the type of washing, and even short periods of exposure were sufficient to reveal differences in metal depositions between the two sites.

Introduction

In the last thirty years, bryophytes were extensively utilized for assessing the atmospheric deposition of heavy metals and/or radionuclides. Many surveys based on bryophytes were carried out at different geographic scales, and several reviews on this subject are available (e.g. Tyler 1970, 1990, Manning & Feder 1980, Maschke 1981, Martin & Coughtrey 1982, Grodzinska 1982, Rao 1982, Brown 1984, Puckett 1988, Nimis 1990, 1996). The results of intercalibration studies show a good agreement between measurements of heavy metals in mosses and in artificial deposimeters (e.g. Rühling & Tyler 1969, 1970, Tyler 1971, Tyler *et al.* 1983, Pilegaard 1979, Rühling 1985, Rühling *et al.* 1987).

Many bryophythes efficiently entrap dust particles thanks to their peculiar morphology, the absorption of mineral elements being favoured by a large area/volume ratio and by a low surface resistance to ion uptake (Brown 1982, 1984). Bryophytes lack a protective cuticle and thickened epidermal cell walls, and their tissues are easily permeable to water and minerals, including metal ions. Due to the absence of a root system, mineral nutrition chiefly derives from wet and dry deposition of particles and soluble salts. The substrate is of almost no importance in the nutrition of pleurocarpous mosses and of little importance in most epiphytic mosses (Tamm 1953, Rasmussen & Johnsen 1976, Rasmussen 1978). However, in certain bryophytes, such as terricolous acrocarpous mosses, an uptake of metals from the substratum seems to occur, mainly with rising capillarity water (*e.g.* Shimwell & Laurie 1972); such forms are less suitable as deposition monitors. The efficiency in particulate trapping mainly depends on features such as the rate of surface hydration, wet bryophytes showing greater efficiency than dry ones. Furthermore, metal levels can be influenced both by canopy cover (Rinne & Barclay-Estrup 1980) and by the sampling position with respect to tree crowns. Metal elements in anthropic or industrialized areas are supposed to be mainly deposited on the surface of bryophytes as particulate matter (Brown 1982, Brown & Bates 1990, Tyler 1990), and according to Brown (1982), bryophytes are morphologically more than chemically suitable to accumulate elements. Their high water retention capacity, furthermore, reduces the loss of entrapped particles by precipitation (Brown 1982).

Levels of metal accumulation recorded in mosses, usually greatly surpassing those of vascular plants (Czarnowska & Rejement-Grochowska 1974), were also attributed to the cation exchange capacity of moss cell walls (Rühling & Tyler 1970). Ion exchange phenomena were shown to be rapid and passive, and the cation exchange capacity of bryophytes, mainly *Sphagnum*, is closely correlated with the plants content of unesterified polyuronic acid molecules (Clymo 1963, Spearing 1972).

The distribution of elements within the moss tissue is important in understanding tolerance mechanisms, and in planning adequate monitoring strategies. Elements can be found in four possible locations: a) as trapped particulate among the shoots, leaves and rhizoids; b) dissolved in the extracellular solution on the outer surface of the plants, c) within the matrix of the cell walls, bound to the exchange or chelating sites of cell walls (intercellular); d) or as intracellular, soluble or insoluble material in the cytoplasm and vacuoles (Brown 1982, 1984, Brown & Bates 1990). In the first three cases the uptake is passive, while in the last one it is the result of an active biological process, using transport sites through the membrane with various degrees of selectivity.

The use of mosses for monitoring heavy metal deposition is simple and cheap. However, mosses are often absent or scarce in urban areas. For this reason, many authors developed transplants methods. Goodman & Roberts (1971) introduced logs covered with *Hypnum cupressiforme* from an uncontaminated area into an industrial area in Wales, obtaining very high accumulation rates. The transplants died after some weeks but they continued to accumulate metals after death. Pilegaard (1979) found that metal accumulation in exposed samples of *Dicranowesia cirrata* growing on their substratum is linearly correlated with atmospheric deposition. The main problem with transplants is that the samples often cannot survive for long periods in the new habitats, partly due to the adverse local climate, or to severe pollution conditions. Although mosses accumulate heavy metals equally well after death, dead transplants easily start to disintegrate. In areas where the use of indigenous mosses is not possible, exposure of standardized samples, *e.g.* moss bags, is more advisable.

The moss-bag technique was developed from transplants experiments with *Hypnum cupressiforme* by Goodman & Roberts (1971). Later authors have usually preferred species of *Sphagnum*, because of its strong resistance to dessication and its high rehydration capacity. Illustrations of the moss bag technique are available in Little & Martin (1974), Ratcliffe (1975), Temple *et al.* (1981), Martin & Coughtrey (1982) and Brown (1984). Moss bags consist of a mesh or grid, generally made of nylon, containing moss shoots whose initial metal content is known; these are often pretreated by distilled water - or acid solution - washing. The bags are suspended in some ways for variable periods in suitable sites before collection and chemical analysis. The main advantages of this technique are the availability of large amounts of material, the uniform and well-defined exposure periods, the possibility of detecting deposition patterns even in "moss desert" areas, thanks to the flexibility of site selection and the high number of sampling sites. Many authors used moss bags to assess deposition patterns of heavy metals linked to industrial emissions (Goodman & Roberts 1971, Little & Martin 1974, Ratcliffe 1975, Gill *et al.* 1975, Cameron & Nickless 1977, Goodman *et al.* 1977, Hynninen 1986); urban emissions (Muskett 1976, Mäkinen 1977), and traffic (Crump & Barlow 1980, Temple *et al.* 1981).

There are variable options in the type of treatment, size and shape of the bags, processing of moss shoots, and habitats in which they are utilized. Some methods used for cleaning bryophyte material and the problems involved are discussed by Brown (1982). A particularly controversial point is still represented by the type of washing: mosses are usually washed in order to remove dust or soil particles and to detect control values of metal contents before exposure. Distilled water removes particles trapped on the surface of the moss, and soluble ions (*e.g.* Na, Mg, K, Ca, etc) accumulated in the intercellular spaces (Bates 1975, Woollon 1975). Acid-washing causes the realese of ions linked to polyuronic molecules of cell walls, which are substituted by protons (Goodman *et al.* 1977). This kind of treatment kills the moss, and hence the subsequent metal uptake is purely passive, but it should enhance the uptake capacity of exposed samples by increasing metal leaching. Comparisons with material lacking the high cation exchange capacity of mosses, as cotton wool, showed that this is much less efficient (Roberts 1972, Mäkinen 1977, Keller 1974). Many authors recommend exposure for relatively short periods, to avoid saturation of exchange sites, but most reports show a linear increase of metal concentrations with time during constant emission periods. Many authors carried out methological studies in order to propose a standard method, which is necessary if absolute deposition rates are to be obtained (*e.g.* Little & Martin 1974, Gailey & Lloyd 1986a, b, c, Kelly *et al.* 1987). No general agreement, however, has been hitherto reached concerning pretreatment of material, density, size, way and time of exposure of the samples.

The previously discussed methodological problems, and the recent, increasing interest in moss methods by Italian authors, led us to undertake a methodological study dealing with the moss bag technique. The present paper particularly concerns three main aspects of data variability: a) variability of metal content in samples collected in natural areas; b) effects of different washing treatments on the metal content of samples, and, c) uptake capacity of exposed moss bags.

Materials and Methods

This study was carried out using *Hypnum cupressiforme* var. *filiforme* Brid., a taxon widespread all over Italy. The epiphytic variety *filiforme* was choosen, as it is less affected by contamination from soil than terricolous or saxicolous varieties. *Hypnum cupressiforme* accumulates most metals from atmospheric deposition, and only a small share is absorbed from tree bark (Rasmussen & Johnsen 1976); Rasmussen (1978) did not find any relationship between metal contents in samples of *H. cupressiforme* and in the bark on which they grew.

The original samples were collected in a dolina near the small village of Basovizza, at ca 400 m in the Karst of Trieste (NE Italy). This area is far from important urban and industrial settlements, and, according to previous bioindication studies, it is unaffected by relevant pollution phenomena (Castello *et al.* 1995). The natural vegetation consists of a mixed deciduous forest dominated by *Quercus cerris*, *Q. petraea* and *Carpinus betulus* (*Asaro-Carpinetum betuli*, *Seslerio-Quercetum petraea*, Poldini 1989).

The variability of metal contents of *H. cupressiforme* carpets growing in the natural site was studied comparing data from individual carpets with those from composite samples, obtained from a mixture of several individual carpets. Individual samples were collected at different heights, from 10 to 120 cm from the ground, on five trunks of *Quercus*, and separately placed in envelopes. For the preparation of composite samples, several moss carpets were collected from different trees, avoiding the basal parts of trunks, and carefully mixed together. All samples were cleaned in the laboratory from soil particles, without separating the brown, old parts of the stems from the green ones, except for basal, very old and necrotic parts, which were strongly incrusted with soil material. 10 individual samples and 10 composite samples taken from the mixed material were used for measuring metal concentrations. These samples were not submitted to washing treatments.

The study of the effects of different washing types on metal ion content and uptake capacity of mosses was carried out on the mixed material: half of it was submitted to 7 consecutive washings with distilled water, lasting from 5 to 20 minutes; the remaining material was washed in a 1% HNO_3 acid solution (Hynninen 1986) for 1 hour, and then shortly washed for five times with distilled water. After washing, all samples were air-dried. Concentration values for 10 water-washed and 10 acid-washed samples were compared with those of the 10 composite unwashed samples, *i.e.* with the natural metal content of the mosses. These values represent the control data for assessing metal accumulation rates in the remaining material, which was used for the exposed moss-bags.

Moss bags were prepared as follows: the bag consists of a nylon mesh 10 cm x 10 cm wide, with 1 mm mesh (Davies & White 1981), closed by a nylon wire, forming spherical bags 3-4 cm diam. In each bag 400 mg of moss material were placed. This quantity exceeds the 100-200 mg optimal weight proposed by Gailey & Lloyd (1986a), but it assures enough material for following chemical analyses even if parts of the samples are lost during exposure.

The bags were exposed in two sites with different air pollution conditions: the control site was the same where mosses were collected, the other was located in the industrial zone of Trieste, near an iron works and a high-traffic highway. In each site, 10 water-washed and 10 acid-washed bags were suspended, in order to study the effects of washing on uptake efficiency. The bags were attached in couples of one water-washed and one acid-washed sample at the extremities of a 20 cm long wooden stick. The sticks were suspended on the outermost branches of trees, 1,5-2 m from the ground. Care was taken to eliminate all leaves lying above the bags. The exposure time lasted for eight weeks, from June, 21 to August, 13, 1993, a period characterized by the lowest value of seasonal precipitations. According to Gailey & Lloyd (1986b) an exposure period of 8-9 weeks is the most appropriate to assure high and relatively replicable concentrations for most metals in the samplers.

For the measurement of metal concentrations, ca. 150 mg of each sample were dried with a microwave oven and mineralized in teflon containers at 120° C for 8 hours with concentrated HNO_3 in a pressure decomposition system. The analysis was perfomed by atomic absorption spectrophotometry, using the graphite furnace for Al, Cd, Pb, the air/acetylen flame for Cu, Cr, Fe, Mn, Ni. To check the analytical method, the NBS Standard Reference Material n. 1572 "Citrus Leaves" was used.

Results

Data variability of metal contents in indigenous moss samples collected in natural sites

One of the variables considered in this study is the height from the ground of samples growing on tree boles. The data obtained from the 10 individual samples taken at heights from 10 to 120 cm on different trees are reported in Tab. 1. Althought no strong difference among heights is evident, it is advisable to collect samples at more than 1 m height, as the variability of the data tends to decrease with increasing distance from the ground, probably due to terrigenuos contamination of the lower samples.

Tab. 1 reports the metal concentrations of the 10 composite samples taken from the mixed material collected in the natural site. Fig. 1 compares the distribution of metal content values of individual and composite samples. Data from individual samples have a larger spread, but the mean values of the two sets of samples are similar.

The variability of metal concentrations in individual samples is high for monitoring purposes. Our results, however, show that it is possible to characterize a given site by analyzing just a few samples taken from a mixture of several randomly collected moss carpets. The resulting measurements are close to the mean of many measurements from individual samples.

Effects of treatments on variability of metal contents in moss samples

Tab. 2 shows the metal concentration values of 10 water-washed and 10 acid-washed samples. The mean concentration values of all metals in washed samples are lower than those of unwashed samples (Tab. 1), with the exception of a slightly higher mean value of Cu in water-washed samples.

Both types of washing cause the partial removal of metal ions accumulated by mosses, but with important differences. Acid solution has a remarkable removing efficiency for Cd, Cu and Mn. Water-washed samples have lower mean values of Al, Cr and Fe, and higher values of Cd, Cu, Mn, Ni and Pb (Fig. 2). This difference, statistically tested by the t-test (Tab. 2) is highly significative for Pb, Cu, Fe, Mn and Cd, significative for Ni, and non-significative for Al e Cr; the significance values of Pb and Fe are slightly lower than those of Cd, Cu and Mn. These results can be interpreted on the basis of the different location of metal ions in mosses. Distilled water is known to be less efficient in removing metal ions than acid solution, but the number of repeated treatments has a great importance too. Distilled water shows a higher efficiency in removing Al, Cr, Fe and a lower efficiency for Pb and Ni; these metals are usually linked to atmospheric particles, both from litho- and anthropogenic sources, which are easily removed by repeated washings. The acid solution treatment is very efficient for Cd, Cu and Mn, which are usually associated with the cell wall, and can be easily substituted by protons.

The choice of washing type must be based on the removal efficiency of metal ions and on the resulting variability of the data. The variation coefficient of water-washed samples (Tab. 2) is always lower than that of unwashed samples (with the exception of Pb, Tab. 1), and of acid-washed samples (with the exception of Fe, Tab. 2). Acid-treated samples have a higher variation coefficient for Cd, Cr, Mn and Pb than unwashed ones. It must be however considered that the higher variability in acid-washed samples could be influenced by a higher analitycal error: the acid-washed samples show low and even very low concentration values of metals and the analitycal error exponentially increases as concentration values dicrease.

It can be concluded that althought acid washing is more efficient in leaching metals, it is possible to treat moss material with distilled water as well, but several repeated washings are advisable.

Uptake capacity and variability of exposed samples

The uptake capacity of moss bags at different pollution conditions, and the data variability of exposed moss bags were considered in this experiment. Tabs. 3-4 and Fig. 3 show the concentration values of metals recorded in moss bags after exposure in the two sites.

Samples from polluted site (Tab. 4) show remarkably higher contents of all metals than those

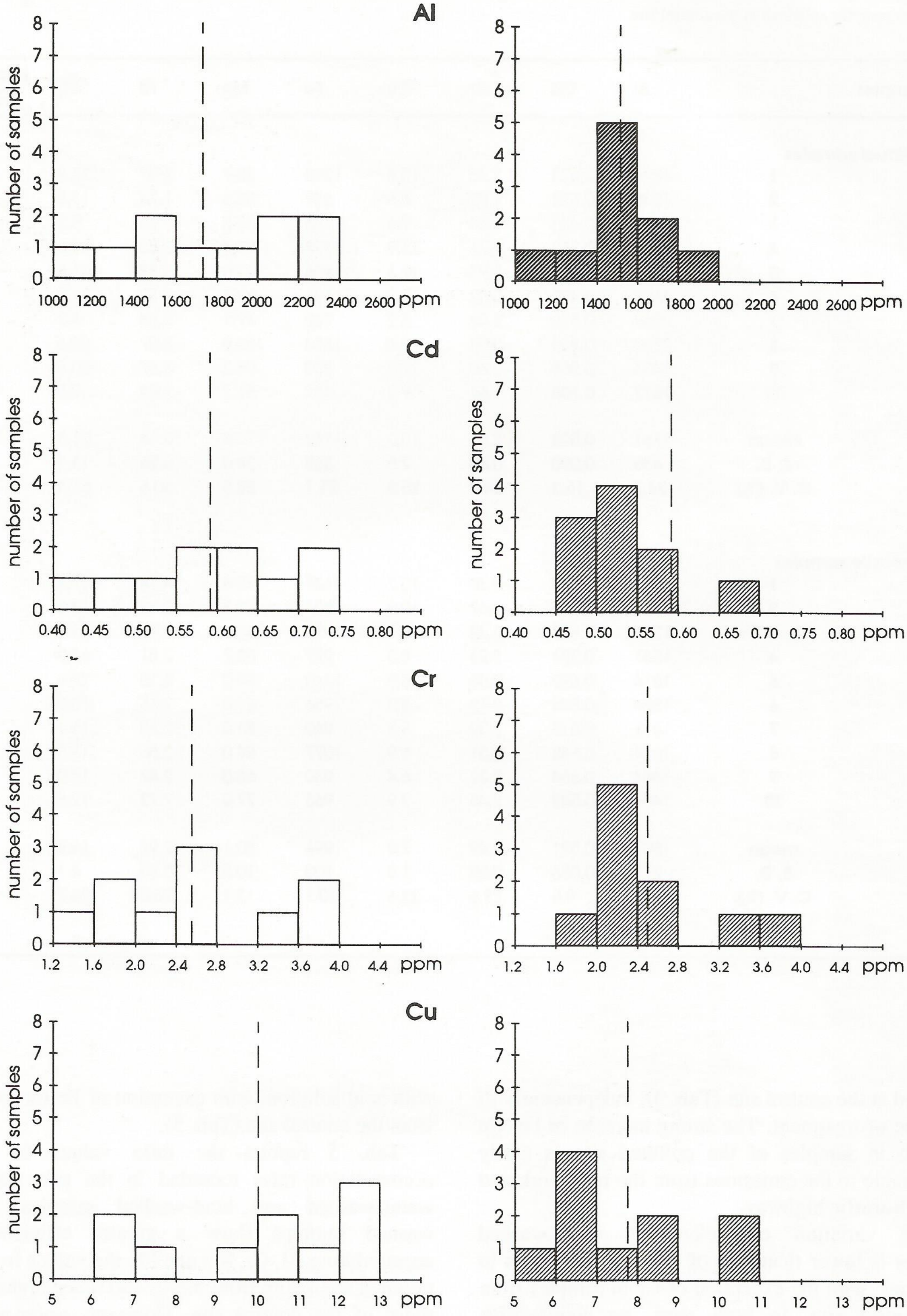
Tab. 1 - Metal concentration, mean, standard deviation values (ppm) and coefficient of variation recorded in 10 individual samples and 10 composite samples collected in the control site.

Samples	Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Heigth (cm)
Individual samples									
1	1825	0.628	2.45	10.8	1290	75.7	3.17	33.8	15
2	1235	0.522	1.63	6.5	899	56.6	1.74	13.8	15
3	1113	0.445	1.54	7.6	784	35.5	1.71	5.2	40
4	2176	0.653	2.71	11.9	1398	93.4	3.43	41.6	50
5	2265	0.720	3.97	12.3	1804	121.0	4.82	31.5	60
6	1600	0.492	3.62	13.0	1196	82.4	3.69	31.9	80
7	2069	0.576	2.02	6.2	762	47.1	3.64	4.2	80
8	2320	0.628	3.54	10.0	1654	36.9	3.07	20.6	90
9	1434	0.558	2.60	12.4	950	95.2	3.67	20.5	120
10	1572	0.704	1.63	9.2	885	81.7	2.44	9.5	120
mean	1761	0.593	2.57	10.0	1162	72.6	3.14	21.3	
S. D.	435	0.090	0.89	2.5	368	28.0	0.96	13.1	
C. V. (%)	24.7	15.2	34.7	25.3	31.7	38.5	30.5	61.4	
Composite samples									
1	1690	0.620	3.30	10.2	1137	75.4	4.14	17.9	-
2	1056	0.528	1.67	10.9	821	69.3	2.19	12.3	-
3	1531	0.587	3.72	5.1	926	94.8	4.75	13.9	-
4	1581	0.721	2.23	8.3	997	82.7	2.81	17.9	-
5	1814	0.599	2.45	6.9	1140	98.0	3.10	25.9	-
6	1524	0.544	2.12	6.9	956	69.0	2.35	18.9	-
7	1411	0.618	2.39	8.5	960	83.0	2.65	16.1	-
8	1674	0.588	2.31	6.9	1077	84.0	2.55	18.5	-
9	1384	0.554	2.32	6.4	930	68.0	2.42	14.0	-
10	1401	0.549	2.43	7.9	963	77.0	2.72	12.5	-
mean	1507	0.591	2.49	7.8	991	80.1	2.97	16.8	
S. D.	211	0.056	0.59	1.8	100	10.5	0.83	4.1	
C. V. (%)	24.2	9.5	23.6	22.6	10.1	13.1	28.0	24.2	

exposed at the control site (Tab. 3), independently of the type of treatment. The strong increase of Fe, Cu and Pb in samples of the polluted site is easily attributable to the emissions from the iron work and the high-traffic highway.

The variation coefficient of water-washed samples is lower than that of acid -washed ones in both sites, with the exception of Cr in samples from the control site. In both sites, no significative difference in uptake capacity was found between bags treated with distilled water and those treated with acid solution, with exception of Fe for samples from the control site (Tab. 5).

Tab. 5 reports the ratio values of mean accumulation rates recorded in the two sites for water-washed and acid-washed samples. Acid-washed samples show a greater efficiency in accumulating Al, Cr, Mn and Pb, the values from the polluted site being from 5.5 to 460 times higher than those of the control site. However, water-washed samples prove to be sensible enough to reveal the different pollution conditions.


Al
Cd
Cr
Cu
number of samples
1000 1200 1400 1600 1800 2000 2200 2400 2600 ppm
0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 ppm
1.2 1.6 2.0 2.4 2.8 3.2 3.6 4.0 4.4 ppm
5 6 7 8 9 10 11 12 13 ppm

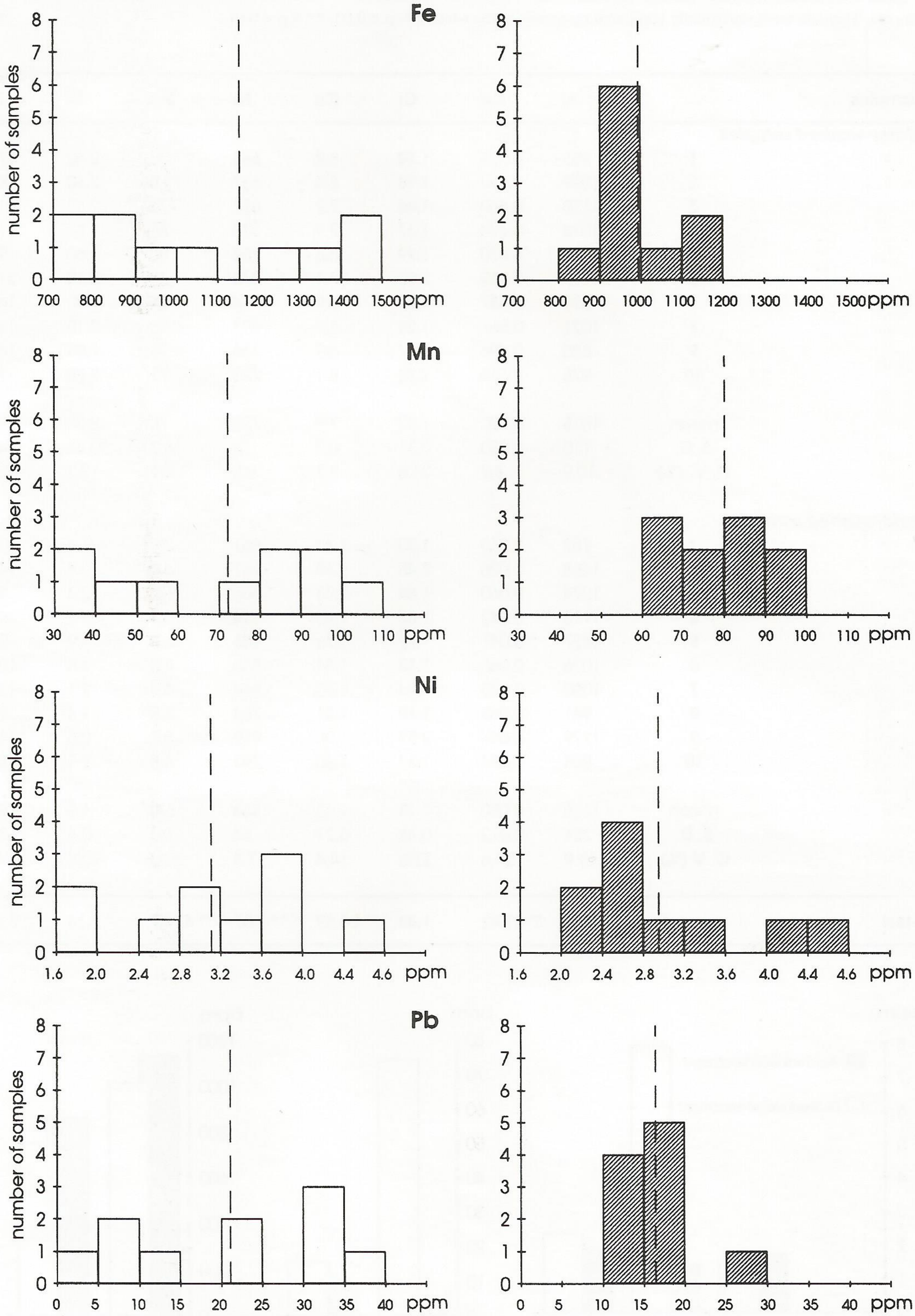


Fig. 1 - Distributions of metal concentration values recorded in 10 individual samples (white bars) and in 10 composite samples (dashed bars). Mean values are reported as dotted lines.

Tab. 2 - Metal concentration, mean, standard deviation values (ppm) and coefficient of variation recorded in water-washed and acid-washed moss samples. The data were statistically compared by means of the t-test. * = p < 0.05, ** = p < 0.01.

Samples	Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb
Water-washed samples								
1	955	0.528	1.39	8.8	683	78	2.60	17.9
2	1029	0.493	1.98	8.4	837	77.8	2.60	18.9
3	1120	0.560	1.46	7.2	858	76	2.60	21.8
4	1078	0.474	1.37	7.9	670	66	1.80	17.8
5	1140	0.570	1.29	8.6	754	82	2.60	9.3
6	1137	0.569	2.00	8.1	870	78	3.10	21.7
7	811	0.437	1.63	6.9	714	67	1.90	14.3
8	1021	0.561	1.24	8.2	803	76	2.10	13.3
9	885	0.496	1.07	6.9	736	73	1.96	16.4
10	975	0.516	1.30	8.1	725	71	2.20	7.2
mean	1015	0.520	1.47	7.9	756	74	2.35	15.9
S. D.	110	0.050	0.31	0.7	72	5.2	0.42	4.9
C. V. (%)	10.9	8.7	21.0	8.7	9.5	6.9	17.7	30.7
Acid-washed samples								
1	782	0.063	1.30	1.40	851	3.9	1.4	10.3
2	1468	0.025	2.45	1.69	957	6.9	2.4	16.9
3	1274	0.060	1.84	1.43	866	6.1	2.3	9.1
4	1115	0.042	1.62	1.48	894	5.3	2	4.5
5	1427	0.041	1.72	1.76	956	4,.2	1.9	5.2
6	1076	0.060	1.33	1.54	840	4.6	1.3	10.8
7	1080	0.045	1.94	1.26	844	4.9	2.1	12.2
8	941	0.063	1.19	1.31	764	3.5	1.4	9.5
9	1279	0.047	2.57	1.05	919	5.7	2.5	11.6
10	904	0.054	1.37	1.40	792	4.8	2.1	8.7
mean	1135	0.050	1.73	1.43	868	5.0	1.9	9.9
S. D.	226	0.012	0.48	0.21	64	1.0	0.4	3.5
C. V.(%)	19.9	24.6	27.6	14.4	7.4	20.8	22.4	35.65
t-test	1.50	** 41.42	1.44	** 28.69	** 3.37	** 41.79	* 2.13	** 3.14

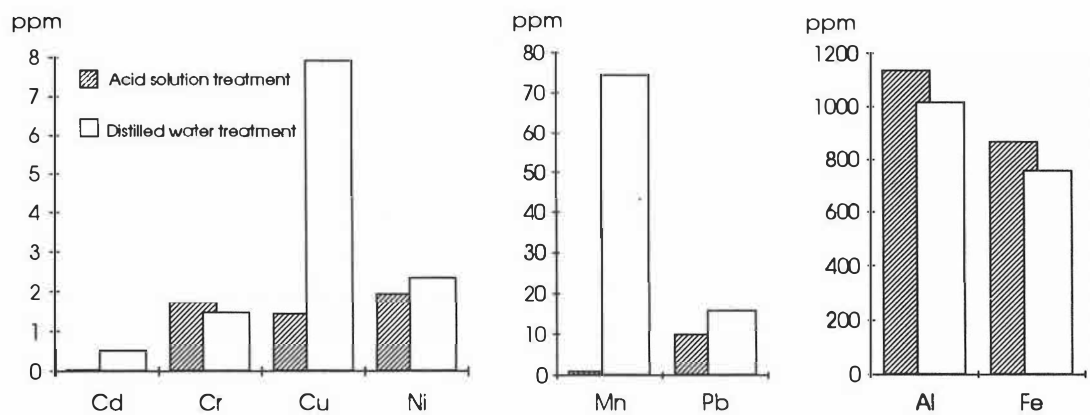


Fig. 2 - Mean metal concentration values recorded in 10 moss samples washed with distilled water (white bars) and in 10 samples treated with acid solution (dashed bars).

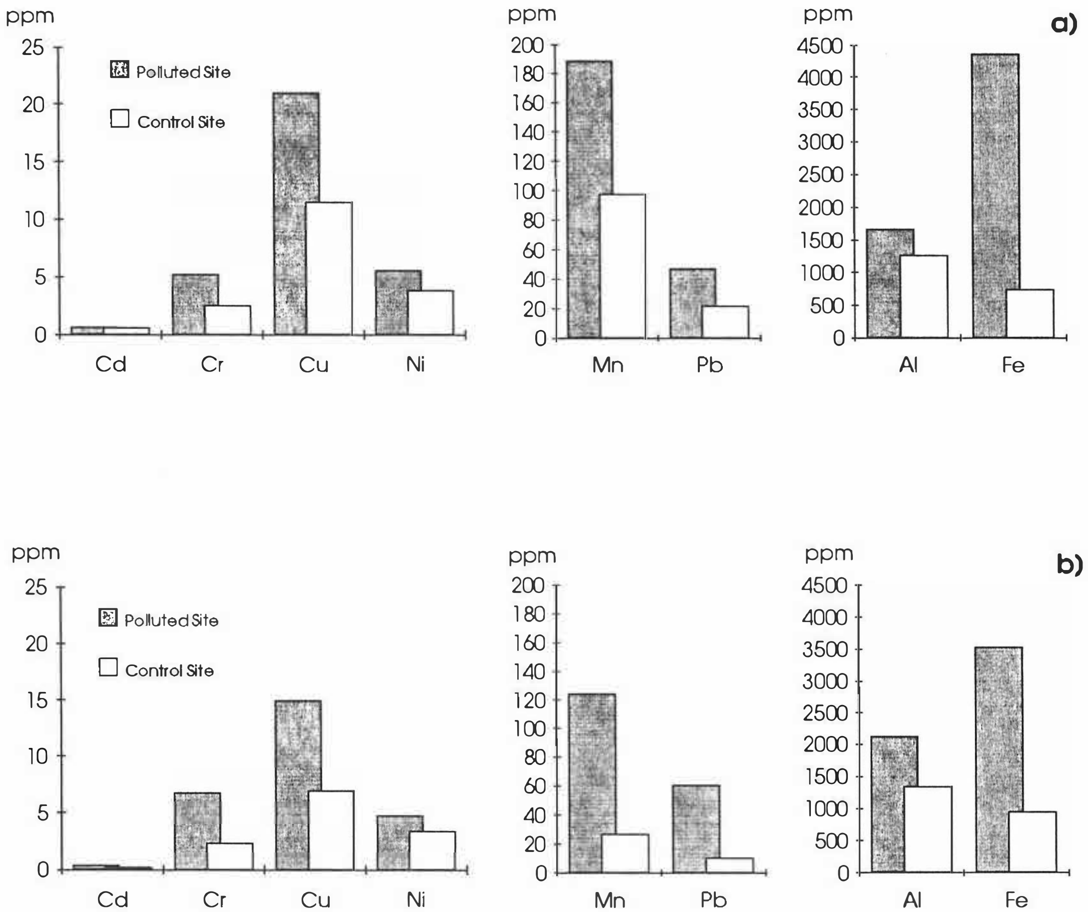


Fig. 3 - Mean metal concentration values recorded in acid-treated (a) and water-treated (b) moss-bags exposed at the control site (white bars) and at the polluted site (dashed bars).

Conclusion

The evaluation of the variability of metal concentrations in individual samples collected in natural areas is important for designing adeguate sampling strategies of monitoring studies based on indigenous and on transplanted mosses, or moss bags (see also Gailey & Lloyd 1986a, b, c). Although biological data of metal contamination are usually very variable, our results show that it is possible to characterize a given sampling site by considering just a few moss samples made up of randomly collected and thoroughly mixed material. Attention, however, must be paid to terrigenous contamination.

The cleaning and washing treatments of moss material represent the most important and critical aspects of the moss bag technique. Distilled water and acid solution act in different ways: the results of this study show that acid washing, a high-impact process that kills the mosses, is highly efficient in removing metal ions usually associated with the cell walls, but produces more variable sets of data. These results differ from those obtained in a similar work by Gailey & Lloyd (1986c), who report a lower variation coefficient in acid-washed samples: the discrepancy could be explained by the efficiency of the acid solution in leaching metals, which could produce variable contents in exchangeable metals, higher degree of difficulty of analitycal depending on different acid concentrations or on the different

Tab. 3 - Metal concentration, mean, standard deviation values (ppm) and coefficient of variation recorded in exposed moss-bags at the control site. Samples are distinguished by the different type of treatment.

Samples		Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb
Water-washed samples									
	1	1772	0.687	6.01	21.6	3766	200	5.22	50.8
	2	1762	0.711	6.45	19.1	4090	181	4.65	53.8
	3	1690	0.826	6.79	23.2	4315	196	5.21	56.7
	4	1674	0.745	3.26	24.0	5528	217	5.72	46.0
	5	1496	0.793	5.19	20.6	4727	192	5.61	33.4
	6	1723	0.533	5.34	19.0	4385	178	6.89	45.1
	7	1540	0.483	3.50	17.9	4003	150	4.9	46.2
	8	1608	0.496	5.49	18.0	4129	158	6.4	41.2
	9	1724	0.749	5.45	23.0	4597	194	5.5	46.8
	10	1646	0.701	4.76	23.4	4036	221	5.5	48.0
	mean	1664	0.672	5.22	21.0	4358	189	5.6	46.8
	S. D.	92	0.124	1.14	2.4	502	23	0.7	6.5
	C.V. (%)	5.5	18.4	21.9	11.3	11.5	12.1	12.1	13.9
Acid-washed samples									
	1	2202	0.385	5.73	17.0	4166	138	4.76	65.1
	2	2147	0.434	5.37	17.7	4063	131	5.02	61.5
	3	2349	0.455	6.59	18.8	4848	161	5.84	81.9
	4	2270	0.317	9.29	15.9	3504	133	4.74	55.2
	5	2012	0.271	10,.2	12.7	3173	122	6.47	58.8
	6	2014	0.239	9.73	13.1	3330	108	5.56	49.8
	7	2014	0.306	5.27	13.7	2930	119	3.56	53.3
	8	1939	0.507	7.91	15.1	3040	111	4.49	49.9
	9	2288	0.278	5.69	12.7	3242	109	3.53	64.8
	10	1992	0.242	5.35	12.6	2963	108	3.43	64.6
	mean	2123	0.343	6.77	14.9	3526	124	4.74	60.5
	S. D.	147	0.095	1.76	2.3	632	17	1.03	9.6
	C. V. (%)	6.9	27.8	26.1	15.5	17.9	13.8	21.8	15.8

durations of the washing processes; the determination of small quantities of elements must be considered.

Metal ions, and above all those associated on particles, are shown to be highly released by repeated washings with distilled water.

The differences in metal concentrations between samples exposed in non-polluted and polluted sites demonstrate that moss bags are able to detect, even after short exposure periods, different degrees of pollution. The uptake capacity and the efficiency of moss bags seem to be only weakly affected by the type of washing.

The treatment of moss material with several repeated washings with distilled water proved to be an effective alternative to acid washings in monitoring studies by moss bags.

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Tab. 4 - Metal concentration, mean, standard deviation values (ppm) and coefficient of variation recorded in exposed moss-bags at the polluted site. Samples are distinguished by the different type of treatment.

Samples	Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb
Water-washed samples								
1	1350	0.633	3.60	12.6	680	86	3.96	18.0
2	1293	0.639	3.22	13.1	741	120	4.27	28.1
3	1198	0.579	4.52	11.9	724	111	5.00	24.5
4	1234	0.575	3.88	14.7	694	102	4.10	22.1
5	1388	0.509	1.98	12.3	699	93	3.30	25.1
6	1280	0.683	1.61	14.2	727	110	3.10	21.6
7	1220	0.507	2.30	8.3	685	95	3.33	23.8
8	1204	0.599	1.41	7.0	797	74	4.94	16.6
9	1174	0.559	1.45	12.3	826	93	3.81	19.9
10	1235	0.565	1.77	8.8	833	89	2.27	18.9
mean	1258	0.585	2.57	11.5	741	97	3.81	21.9
S. D.	69	0.056	1.13	2.6	58	14	0.84	3.6
C. V. (%)	5.5	9.6	44.1	22.5	7.8	13.9	22.1	16.4
Acid-washed samples								
1	1369	0.144	2.46	4.2	1041	16.2	2.97	9.5
2	1266	0.131	2.33	4.5	909	17.6	2.46	9.1
3	1308	0.199	2.54	8.9	954	25.4	3.81	6.4
4	1361	0.122	2.70	4.2	988	9.4	7.50	13.3
5	1285	0.139	2.28	8.0	1000	28.8	2.85	8.8
6	1279	0.120	2.14	5.2	902	20.8	2.26	11.9
7	1356	0.292	2.39	12.9	968	60.2	3.32	11.1
8	1643	0.345	2.41	9.3	793	24.2	3.40	11.3
9	1273	0.179	2.17	6.5	904	40.9	2.43	7.4
10	1273	0.112	2.17	5.2	1033	21.5	2.87	11.1
mean	1341	0.178	2.36	6.9	949	26.5	3.39	10.0
S. D.	113	0.080	0.18	2.8	75	14.5	1.52	2.1
C. V. (%)	8.4	44.7	7.6	40.8	7.9	54.7	45.0	21.3

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Tab. 5 - Mean concentration values (ppm) of moss samples before and after exposure at the control and at the polluted site. Accumulation data of water- and acid-washed moss bags were calculated as the difference between these mean concentration values, and were statistically compared by means of the t-test. ** = $p < 0.01$. The accumulation efficiency of water- and acid-washed moss bags was estimated by calculating the ratio between accumulation rates at the polluted and at the control site.

Samples		Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb
Control Site									
Water-washed samples									
	before exposure (A)	1015	0.520	1.47	7.91	756	74.5	2.35	15.9
	after exposure (B)	1258	0.585	2.57	11.52	741	97.4	3.81	21.9
	accumulation (B-A)	243	0.065	1.10	3.61	-15	22.9	1.46	6.0
	S.D.	180	0.106	1.44	3.27	130	18.7	1.26	8.5
Acid-washed samples									
	before exposure (A)	1135	0.050	1.73	1.4	868	5.0	1.94	9.9
	after exposure (B)	1341	0.178	2.36	6.9	949	26.5	3.39	10.0
	accumulation (B-A)	206	0.128	0.63	5.5	81	21.5	1.45	0.1
	S.D.	339	0.092	0.66	3.0	139	15.5	1.96	5.7
t-test		0.17	0.85	0.51	0.73	** 7.47	0.10	0.01	1.00
Polluted Site									
Water-washed samples									
	before exposure (A)	1015	0.520	1.47	7.9	756	74.5	2.35	15.9
	after exposure (C)	1664	0.672	5.22	21.0	4358	188.7	5.56	46.8
	accumulation (C-A)	649	0.152	3.75	13.1	3602	114.2	3.21	30.9
	S.D.	202	0.174	1.45	3.0	575	27.9	1.09	11.4
Acid-washed samples									
	before exposure (A)	1135	0.050	1.73	1.4	868	5.0	1.94	9.9
	after exposure (C)	2123	0.343	6.77	14.9	3526	124.0	4.74	60.5
	accumulation (C-A)	988	0.293	5.04	13.5	2658	119.0	2.80	50.6
	S.D.	373	0.108	2.24	2.5	697	18.1	1.47	13.1
t-test		1.39	1.09	0.84	0.19	1.81	0.25	0.39	1.95
Accumulation ratio									
Water-washed	(C-A)/B-A)	2.7	2.4	3.4	3.6	233.9	5.0	2.2	5.2
Acid-washed	(C-A)/B-A)	4.8	2.3	8.0	2.5	32.7	5.5	1.9	460.1

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