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METHODS FOR PROCESSING AQUATIC MOSSES USED AS MONITORS OF HEAVY METALS

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Abstract—An evaluation is reported of methods used to prepare mosses for analysis when required for monitoring metal pollution. *Fontinalis antipyretica* and *Rhynchostegium riparioides* taken from the River Hoëgne, Belgium, were used for the study. The stages in preparation studied critically were the storage of the moss after it had been collected from the river, washing, choice of which particular fraction to use and the conditions for final drying prior to digestion. For any one particular treatment, the concentrations of metals analysed (Ca, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb) were usually lower in *Fontinalis* than *Rhynchostegium*. Metal concentrations found as a result of the various treatments differed markedly for some metals, including Zn, Cd and Pb. However no one sequence of methods is ideal for all purposes. Criteria that need to be considered when selecting methods include the time and facilities available and whether the moss is to be used for monitoring long-term or short-term pollution.

INTRODUCTION

The accumulation of heavy metals by aquatic vascular plants and bryophytes growing in contaminated environments has been documented by many authors (e.g. Dietz, 1973; McLean & Jones, 1975; Harding & Whitton, 1978; Burton & Peterson, 1979; Welsh & Denny, 1980). In recent years there has been a slight change in emphasis in such papers from those pointing out that accumulation occurs to those where the plants are actually put to practical use as monitors (e.g. for vascular plants: Ray & White, 1979; Nakada *et al.*, 1979; Franzin & McFarlane, 1980). For some years we have been studying in two independent laboratories the potential and practical use of aquatic bryophytes as monitors of heavy metal pollution in rivers. These studies have been based largely in Belgium (Empain 1973, 1976a, b; Empain *et al.*, 1980; Mouvet, 1980) and Northern England (Whitton & Say, 1975; Say *et al.*, 1981; Wehr *et al.*, 1981; Whitton *et al.*, 1982). A standard "package" of ten plants has been recommended (Whitton *et al.*, 1981) for monitoring purposes in Northern Europe; five of these plants are bryophytes.

The considerable literature on heavy metal accumulation is based on a variety of different practical methods. Marked differences exist, for instance, in methods for collection, pretreatment and analysis; sometimes few or no details of methods are given at all. If the chemical analysis of submerged plants is to be put to routine practical use for monitoring heavy metal contamination, then it is clearly essential to establish the effects of varying all these stages on the final concentrations of metals measured. The present study set out to help establish this for two common mosses, *Fontinalis antipyretica* and *Rhynchostegium riparioides*. It was carried out by subjecting materials collected at the same time to the two complete sequences of methods used in the past in Liège and Durham.

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MATERIALS AND METHODS

Site used for collection

The study was carried out on materials collected from a site on the R. Hoëgne in eastern Belgium (50°33'N, 5°48'E). The site corresponds to that listed as H04 in the study of Descy & Empain (1981). This river drains an area of former mining activity and carries, on occasions, elevated levels of zinc and sometimes also cadmium and lead (Descy & Empain, 1981). The river at this site is fast flowing, open to the sunlight throughout the year and the substratum is composed mainly of boulders, cobbles and pebbles. The mosses were sampled on two occasions (22 October 1980, 13 July 1981); water was collected for analysis on the first of these.

Water chemistry

Water was collected and analysed following the methods outlined in Say *et al.* (1981). The values for chemical variables shown in Table 1 also include means reported for the study by Descy & Empain (1981).

Sampling and collecting mosses

Two species were used: *Fontinalis antipyretica* Hedw. and *Rhynchostegium riparioides* (Hedw.) C. Jens. [Syn. *Eurhynchium riparioides* (Hedw.) Rich., *E. rusciforme* (Br. Eur.) Milde, *Platyhypnidium riparioides* (Hedw.) Dix.]. Choice of material was restricted to plants that were fully submerged and as far as possible subjected to relatively fast current speeds. Five replicates of the particular moss were collected per sample. Each replicate was taken from either a single

Table 1. Representative chemistry for study site. A after Descy & Empain (1981): metals measured in water passed through a 0.4 μm Nuclepore filter; B water collected 22 October 1980: metals measured in water passed through a 0.2 μm Nuclepore filter. All elements as mg l^{-1}

	A	B
pH	7.6	7.6
O_2 (% sat.)	94	—
Conductivity (μmho cm^{-1})	302	210
Alkalinity (meq l^{-1})	2.00	2.02
PO_4–P	0.093	0.027
NO_3–N	2.0	
SO_4–S	27.0	
Cl	10.5	
Ca	38.6	36.7
Mn	0.019	0.02
Fe	0.107	0.04
Cu	0.007	0.005
Zn	0.102	0.139
Cd	0.0019	0.0005
Pb	0.0126	0.001

point or group of plants from a single boulder or very small area of the river bed. The material was first washed several times in river water to remove any obvious accumulated sediment and attached invertebrates; it was then squeezed gently to remove excess water before placing in the appropriate containers. The materials were then used for one of the two standard sequences of method or for detailed investigation of particular stages.

Sequence of methods I—after Empain (1977)

Key features are the use of whole plants, a lengthy washing stage in the laboratory and a relatively low final drying temperature. The original method used for washing has become progressively modified, with automation now enabling large amounts of material to be processed quickly.

Material is first put into paper packets, which are themselves placed loosely in large plastic crates with large slots to permit adequate ventilation. In the laboratory crates of packets are stored at room temperature (approx. 20°C) and once dry (24–36 h) can be kept indefinitely before the washing stage is commenced. When this latter is required, material is re-wetted in 500 ml plastic containers with deionised water. A preliminary bulk washing is done by hand, with deionised water flowing over a plastic sieve (approx. 2 mm mesh) for about 4 min; this removes most of the residual foreign matter, but at the same time any other plant species remaining are removed by hand. The next stage is the use of the automatic washing system (Fig. 1), which can take up to four samples, each of five replicates. This system provides vigorous agitation by rapidly flowing deionised water mixed with compressed air, which causes a jetting action through the compartments. The water then flows out through the holes in the plastic lids. Two of these systems may easily be operated at once. The complete washing sequence for eight samples requires approx. 100 l. of deionised water (or 12.5 l. per sample of five replicates). When the washing sequence is finished, excess water is squeezed from the moss and the material is placed in fresh paper packets and dried at 40°C. Enough material is dried to give a minimum final weight of 250–300 mg.

Sequence of methods II—after Say et al. *(1981)*

Key features are the restriction of the part of the plant used for analysis to apical 2 cm lengths (as reported for the alga *Lemanea:* Harding & Whitton, 1981), the completion of washing before the moss is dried and the use of a relatively high final drying temperature.

Material is first placed in acid-washed polypropylene bottles and stored in ice-cooled refrigerator boxes until returned to the laboratory. As far as possible the material is processed immediately on return, but where this is not feasible (as with most of the samples in the present study) it is left overnight in a refrigerator (5°C). Laboratory preparation is commenced by washing the material thoroughly under a stream of distilled water to further remove visible contaminants and then transferring it to an acid-washed Pyrex crystallisation dish of deionised water. From this, shoots of moss are removed and placed in a Pyrex petri dish of deionised water; here 2 cm apical tips of the shoots are removed using stainless-steel forceps and a scalpel. Sufficient tips are taken to give a final dry weight of 25–50 mg per replicate; in the case of these two mosses about 30 tips usually suffice. The tips are washed in several changes of deionised water and then placed momentarily on a pad of absorbent paper to remove excess water. Finally they are put into acid-washed snap-top vials and dried to constant weight at 105°C.

Comparison of methods at various stages

Further sequences of methods were designed (Table 2) to look more critically at the individual stages (storage, washing, choice of moss fraction, final drying).

Digestion and analysis

The procedure used for digestion and analysis was the same for all sequences of methods studied. Approximately 50 mg dry weight (weighed accurately) of each replicate

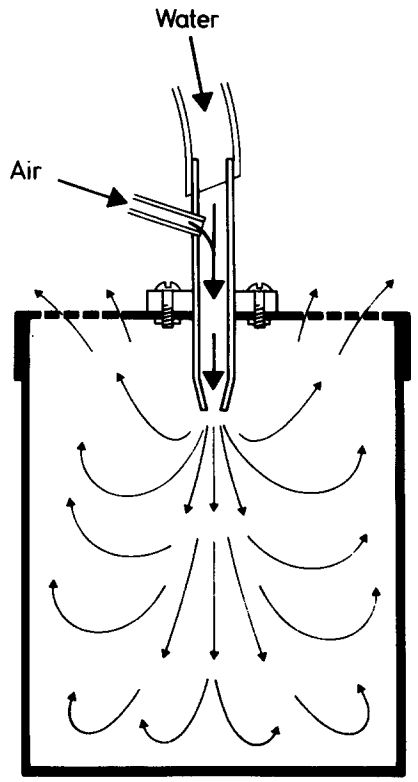

Fig. 1. Construction of automatic bryophyte washing container, showing combined air and water jet for agitation. Perforated lid provides for the flow-through of water and sediments; screws are composed of nylon and all other components of polythene.

Table 2. Sequences used for testing effects of individual stages on metal concentrations: A–F based on material collected 22 October 1981; G–J on material collected 13 July 1981

Stage tested by comparison	Sequence	Stages: Storage	Stages: Washing	Stages: Moss fraction	Temperature for final drying (°C)
Storage	G	Fresh	Automatic	Whole	40
	H	Air dry	Automatic	Whole	40
Washing	A	Fresh	Hand	2 cm tips	105
	B	Fresh	Automatic	2 cm tips	105
	C	Air dry	Automatic	Whole	40
	D	Air dry	Hand	Whole	40
Moss fraction	E	Fresh	Hand	Whole	105
	A	Fresh	Hand	2 cm tips	105
	C	Air dry	Automatic	Whole	40
	F	Air dry	Automatic	2 cm tips	40
Temperature of final drying	I	Air dry	Automatic	Whole	40
	J	Air dry	Automatic	Whole	105
Method after Empain (1977)	C	Air dry	Automatic	Whole	40
Method after Say *et al.* (1981)	A	Fresh	Hand	2 cm tips	105

was placed in an acid-washed Pyrex boiling tube, together with 5 ml concentrated HNO_3 (atomic absorption grade) and the tubes placed in a Tecam dri-block heating mantle. The material was digested at 150°C for 35–45 min or until no more brown fumes of NO_2 were driven off. The digests were made up to 25 ml with deionised water and placed in acid-washed snap-cap vials and stored in a refrigerator (5°C) until analysis. Metals (Ca, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb) were analysed by atomic absorption spectrophotometry (Perkin–Elmer 403) against equivalent molarity, acid matrix-matched standards.

Statistical procedures

Metals in digests which were more than five times greater than the atomic absorption limit were compared statistically; only in the cases of Co and Ni was it found impossible to fulfill this criterion for every sequence of methods.

Individual comparisons of average metal levels in mosses between pairs of methods and between the two species were made using Student's *t*-test, where the null hypothesis (H_0) is $\mu 1 = \mu 2$. As the alternative hypothesis (H_1) is $\mu 1 > \mu 2$ or vice-versa, a one-tailed test was used, where the null hypothesis is rejected if the probability of this occurring strictly due to chance (P) is <0.05. All data were tabulated on computer and solutions calculated using the MIDAS statistical package (Fox & Guire, 1976). This analysis includes a test of the equality of variances of the two means as a requisite to comparing the means themselves. For all those methods which were found to differ significantly the ratio (r) of the two mean metal levels was calculated.

Variability of the sequences of methods was assessed by comparing average coefficients of variation in each. A further comparison was made between separate shoots of whole plants (sequence C: Table 2) and otherwise identical plants where replicates were taken from homogenized material. (Homogenization was carried out by grinding plants to a powder, using an acid-washed mortar and pestle.)

RESULTS

Comparison of methods

A comparison of the concentrations of metals found by sequences of methods A–J in the two mosses is shown in Fig. 2. In general concentrations were higher in *Rhynchostegium riparioides* than in *Fontinalis antipyretica* with any one particular sequence of methods. The concentrations of particular metals not only varied according to treatment used, but the comparative effect of different treatments varied from element to element. At one extreme, Mn in *Fontinalis* ranged from 0.6 to 13.9 mg g^{-1} according to the sequence of methods used, whereas at the other extreme Ca in the same moss ranged only from 5.5 to 9.6 mg g^{-1}. Visual comparison of the results in Fig. 2 does however suggest that the comparative effect of different treatments is quite similar for certain groups of elements, especially Mn, Zn and Cd. Concentrations of all metals considered (Table 3) were significantly greater in both moss species when processed according to the sequence of methods (C in Fig. 2) after Empain (1977) as compared with the sequence of methods (A in Fig. 2) of Say *et al.* (1981).

In order to interpret the reasons for the differences between different sequences of methods, the individual stages were looked at more critically, by comparing pairs of sequences differing at only one stage (Table 2).

Storage

A comparison of metal concentrations found in whole plants stored fresh and slightly chilled (G in Table 2) with whole plants stored air-dry (H) showed higher levels of all metals with the latter treatment (Table 4). In most cases the concentrations were more than twice as high in plants stored air-dry.

Washing

Comparisons of metal concentrations in whole plants washed automatically (C) with those in plants washed by hand (D) showed that all metals except Ca were less in the former, more vigorous, method (Table 5). This effect was not found for *Fontinalis*. A

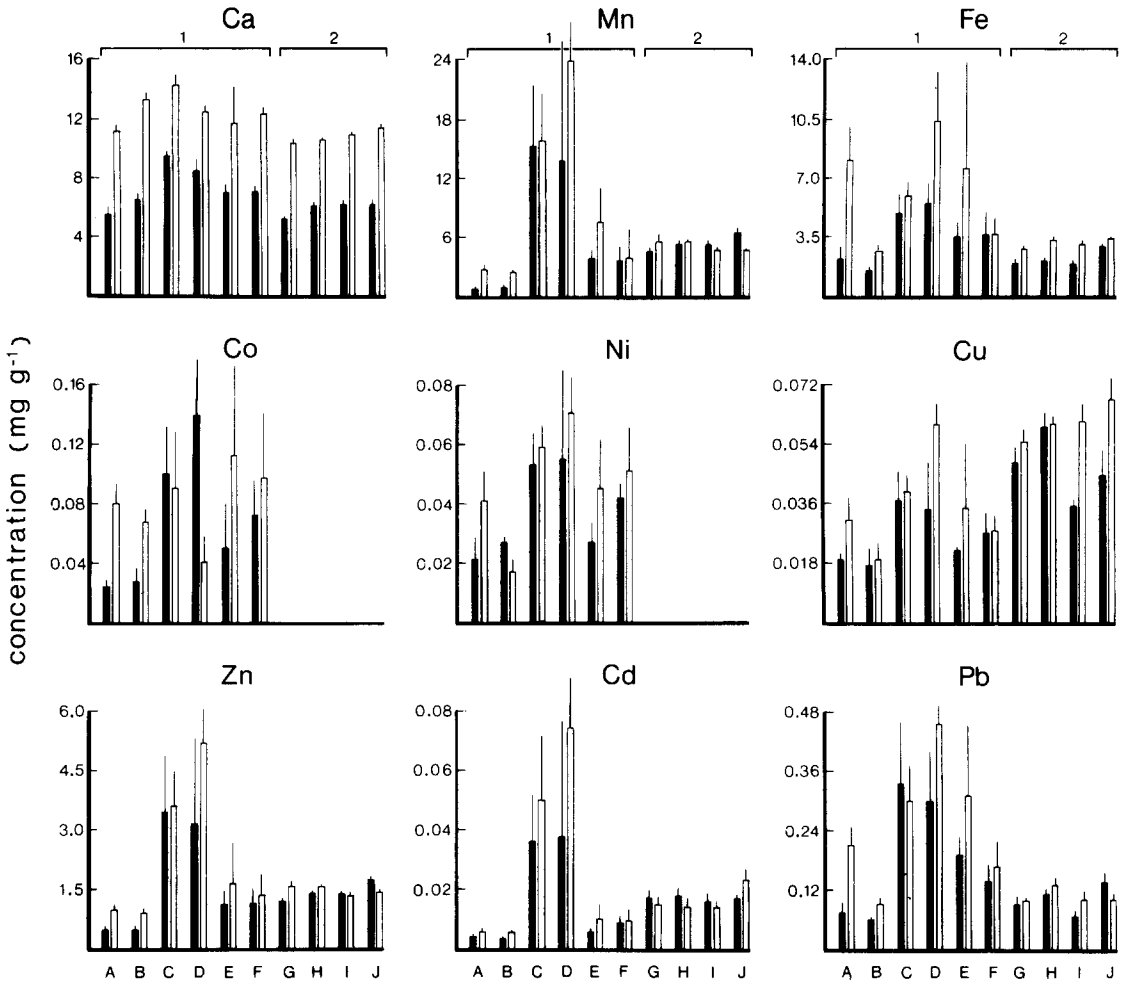

Fig. 2. Metal composition (mg g^{-1}) of *Fontinalis antipyretica* (solid bars) and *Rhynchostegium riparioides* (open bars) with standard deviations.

Table 3. Comparison of concentrations of metals shown by two methods (C vs A) used by previous authors (Empain, 1977; Say *et al.*, 1981); only those examples are included which differ significantly. Dif = differences via one-tailed *t*-test, $P < 0.05$; r = ratio of concentrations

Species		Ca	Mn	Fe	Cu	Zn	Cd	Pb
Fontinalis antipyretica	dif	C > A	C > A	C > A	C > A	C > A	C > A	C > A
	r	1.6	24.1	1.9	1.9	7.5	8.4	4.2
Rhynchostegium riparioides	dif	C > A	C > A	C > A	C > A	C > A	C > A	C > A
	r	1.3	5.8	1.4	1.3	3.4	8.8	1.4

Table 4. Comparison of concentrations of metals shown by two methods (G vs H) to test the effect of storage; only those examples are included which differ significantly. Dif = differences via one-tailed *t*-test, $P < 0.05$; r = ratio of concentrations

Species		Mn	Fe	Ni	Co	Cu	Zn	Cd	Pb
Fontinalis antipyretica	dif	H > G	H > G	H > G	H > G	H > G	H > G	H > G	H > G
	r	2.6	2.1	2.7	3.5	1.8	2.4	3.0	1.8
Rhynchostegium riparioides	dif	H > G	H > G	H > G	H > G	H > G	H > G	H > G	H > G
	r	2.6	2.3	3.4	3.6	1.9	2.1	2.9	1.9

Table 5. Comparison of concentrations of metals shown by two pairs of methods (C vs D; A vs B) to test effect of washing; only those examples are included which differ significantly. Dif = differences via one-tailed *t*-test, $P < 0.05$; r = ratio of concentrations

Species		Ca	Mn	Fe	Cu	Zn	Cd	Pb
Whole plants								
Fontinalis antipyretica	dif	C > D						
	r	1.2						
Rhynchostegium riparioides	dif	C > D	D > C	D > C	D > C	D > C	D > C	D > C
	r	1.1	1.5	1.7	1.5	1.4	1.4	1.5
2 cm tips								
Fontinalis antipyretica	dif	B > A		A > B				A > B
	r	1.1		1.7				2.7
Rhynchostegium riparioides	dif	B > A		A > B	A > B			A > B
	r	1.2		3.1	1.6			2.2

similar comparison was made using 2 cm tips (B vs A). Automatic washing brought a reduction in the concentration of Fe and Pb in 2 cm tips of both mosses, but had little effect on other metals (Table 5).

Moss fraction

Concentrations of metals in whole plants of both species were significantly higher than in 2 cm tips (Table 6). The differences were significant in all cases when automatic washing was included in the sequence of methods, with the differences being particularly pronounced for Mn, Zn and Cd. The differences were less pronounced when washing was carried out by hand.

Temperature of final drying

Whole plants dried at 105°C (J) had significantly higher metal concentrations than those dried at 40°C (I) for both mosses (Table 7). However the differences as expressed by the ratios in most cases were not large.

Variability

Average coefficients of variation (expressed as percentage) for various methods are given in Table 8. For any particular stage, no one method was significantly more variable than another. With one exception, this applied also to comparisons of complete sequences of methods. The exception is provided by the comparison of sequence E with A (hand-washed whole plants vs 2 cm tips), where the combined effects of all stages (including final analysis) lead to the variability of E being greater than that of A for *Rhynchostegium* only.

One further study was carried out in addition to those summarized in Table 2. This involved a comparison of the coefficients of variation of metals in five

Table 6. Comparison of concentrations of metals for two pairs of methods (C vs F; A vs E) to test effect of choice of moss fractqn; only those examples are included which differ significantly. Dif = differences via one-tailed *t*-test, $P < 0.05$; r = ratio of concentrations

Species		Ca	Mn	Fe	Cu	Zn	Cd	Pb
Fontinalis antipyretica	dif	C > F	C > F		C > F	C > F	C > F	C > F
	r	1.3	3.7		1.4	2.8	4.2	2.4
Rhynchostegium riparioides	dif	C > F	C > F	C > F	C > F	C > F	C > F	C > F
	r	1.2	3.6	1.6	1.4	2.6	5.4	1.8
Fontinalis antipyretica	dif	E > A	E > A			E > A		E > A
	r	1.3	5.8			2.4		2.4
Rhynchostegium riparioides	dif		E > A					
	r		2.8					

Table 7. Comparison of concentrations of metals shown by two methods (I vs J) to test the effect of final drying temperature; only those examples are included which differ significantly. Dif = differences via one-tailed *t*-test, $P < 0.05$; r = ratio of concentrations

Species		Ca	Mn	Fe	Cu	Zn	Cd	Pb
Fontinalis antipyretica	dif		J > I	J > I	J > I	J > I		J > I
	r		1.2	1.5	1.3	1.2		2.0
Rhynchostegium riparioides	dif	J > I	J > I	J > I	J > I		J > I	
	r	1.1	1.1	1.1	1.1		1.6	

Table 8. Comparison of average ($\bar{x}$ of 7 metals) coefficients of variation for metal concentrations found in selected methods; only those differences which are significant are included. Dif = differences via one-tailed *t*-test, $P < 0.05$; r = ratio of coefficients of variation

Methods compared	*Fontinalis antipyretica* Mean Coefficient of variation	Dif r	*Rhynchostegium riparioides* Mean Coefficient of variation	Dif r
C vs A	32 ± 15%		24 ± 13%	
	24 ± 15%		18 ± 8%	
D vs C	32 ± 15%		24 ± 13%	
	57 ± 39%		15 ± 8%	
A vs B	13 ± 10%		13 ± 8%	
	23 ± 15%		18 ± 8%	
C vs F	32 ± 15%		24 ± 13%	
	28 ± 12%		36 ± 24%	
E vs A	17 ± 8%		60 ± 22%	B > C 3.3
	24 ± 15%		18 ± 8%	

individual shoots of a clump of *Fontinalis antipyretica* with those of five replicate samples of homogenized material from the same clump. As might be expected, the coefficients of variation were always less with homogenized material (Table 9).

DISCUSSION

It is clear that the various combinations of methods used at the different stages of treatment lead to results which differ, sometimes markedly so. A striking example of this is illustrated when a direct comparison is made between the sequence of methods after Empain (1977) and the sequence after Say *et al.* (1981) where for some metals (Zn, Cd, Pb) large differences in concentrations were found in the mosses (Table 3). This emphasizes the care which should be taken when comparing the metal compositions of aquatic plants from studies reported in the literature. It also emphasises the need for detailed descriptions of all the methods used in preparing accounts of the metal composition of aquatic plants.

Different methods of storage have a pronounced effect on metal concentrations in samples of these two mosses which are otherwise treated in the same way. Material air-dried immediately after collection from the river and stored air-dry had much higher concentrations of metals than material kept fresh and slightly chilled (Table 4). The higher concentrations in dried mosses may result from the drying process reducing the amount of surface bound metal removed in the later washing stages. Older parts of mosses (included in the whole plant, but not most 2 cm tips) frequently have coatings of manganese and iron oxides on the leaves and stems; such oxides in alluvium are known to increase the adsorption and co-precipitation of other metals (e.g. Cu, Zn, Pb: Robinson, 1981). If the material is dried before treatment, it may reduce the efficiency of removal of these tightly bound coatings during washing. In contrast to this possibility Brown & Buck (1979) have shown that soluble ions may leak from bryophytes on rehydrating after desiccation, although sometimes these ions become bound to the cell wall exchange sites. If mosses are kept moist until washed in the laboratory, there is probably an increased likelihood of removing both surface coatings of oxides and also metals loosely bound to the cell walls.

The more vigorous automatic washing technique usually resulted in lower final concentrations of metals as compared with hand washing (Table 5). It was evident visually that the former method removed more of the loosely bound material attached to the leaves and it probably also removed metals loosely bound to the cell walls. This effect of washing was more pronounced with *Rhynchostegium* than *Fontinalis* and may reflect the differences in binding of material to the cell walls of the two mosses. The effect of automatic washing on metal concentrations was less evident with 2 cm tips than with whole plants; this may be associated with the lack of visually obvious coatings on the leaves of the former. Of the metals analysed only iron and, to a lesser extent, lead were significantly lower in the tips by vigorous washing.

Table 9. Comparison of coefficients of variation for selected metals in whole plants of *Fontinalis antipyretica* with replicates ($n = 5$) collected as individual whole shoots vs replicates from homogenized material

	Ca	Mn	Fe	Cu	Zn	Cd	Pb
Individual shoots (%)	9.4	56	30	36	44	58	41
Homogenized plants (%)	2.6	42	24	26	31	47	27

The choice of fraction has a considerable effect on the final metal concentrations in these mosses. Analyses based on whole plants always showed higher values than those based on 2 cm tips (Table 6). This is probably related to several factors. The whole plant includes older parts which have had longer exposure to the metals than young tips. The lowermost parts of plants are covered by coatings of manganese and iron-oxides, which, as mentioned above, presumably bind other metals. Studies on the metal content of 1 cm fractions of the liverwort *Scapania undulata* taken from metal polluted sites have shown that there is usually a progressive increase in metal content (Mn, Fe, Zn, Cd, Pb) on passing from the tip to the base (Whitton *et al.*, 1982). Manganese and zinc have also been shown to increase from tip to base in populations of *Fontinalis antipyretica*, with a high correlation between the concentrations of the two elements (unpublished results).

The differences in the concentrations of metals in plants dried at 40 and 105°C is presumably due to the plants retaining a higher water content at the lower temperature, thereby leading to a lower metal content when expressed per unit dry weight. Experiments by the authors on various populations of both moss species have shown differences in dry weight of 3–10% between the two drying temperatures. These values are similar to the differences in metal content found in the present study (Table 7).

Comments and suggestions

While it is clear that different methods of preparing mosses for analysis lead to different results, it is difficult to state that one method is better than another. In most cases the choice of method depends on the questions to be asked, but other criteria sometimes play a role such as the amount of material available, the laboratory facilities available and possibly also the time allocated to each sample.

Storage. Air-dried samples have the advantage of permitting inexpensive, long-term storage without any pretreatment of the plants after collection. There is no equipment needed in either the field or the laboratory. The generally higher levels of metals in air-dried plants reduces problems connected with analyses close to the detection limit. Air-dried storage of mosses prior to their eventual washing and analysis is especially suited for preliminary surveys involving the collection of plants from many different sites, particularly if these are distant from the analytical laboratory. Short-term storage (air-dry or fresh) is necessary for critical studies where it is important to get the maximum information possible from the sample by relating observations to the results of laboratory studies on uptake and localization of metals.

Washing. Automatic washing is a more rapid means of removing attached matter, including loosely bound deposits on the leaves, but results in a loss of sensitivity through reduction in the total metal content. Hand washing is less damaging to the more delicate plants and is probably adequate for cleaning samples if only the 2 cm tip is being considered. Hand washing requires less deionised water (1.5 l. vs 12.5 l. for five replicates), but takes considerably longer (50 min vs 3 min for five replicates). The construction of automatic washing facilities is probably worth considering in situations where very large numbers of samples (100+) are processed on a single day; obviously this is more likely to be more useful where air-drying is employed, permitting washing to be carried out at long intervals.

Choice of moss fraction. Whole plants have the clear advantages of simplicity in collection and pretreatment and also higher overall metal concentrations. These higher concentrations however probably often include a substantial contribution from the metals accumulated among the oxide coatings on the older parts of the plant. The 2 cm fraction would appear to have greater sensitivity over short-term periods. Accumulation experiments with the alga *Lemanea* (Harding & Whitton, 1981) have shown that sudden increases in metal levels in the water are paralleled by quite rapid increases in metal concentration in 2 cm tips of this plant.

It is suggested that whole plants are suited for broad surveys where what is needed is a rough estimate of the extent of pollution—whether, for instance, a factory discharge has ceased or a new one commenced. Tips of 2 cm are particularly suited for making detailed statistical comparisons of values obtained at one site sampled frequently over a period of time; they are also important when intermittent events need to be monitored, such as when a body of polluted water is suspected of having passed downstream a few hours before the time of sampling.

Although there are inherent problems in sampling and processing river mosses, in only one comparison of sequences of methods was there a significant difference between coefficients of variation. Such variability can arise not only at each stage shown in Table 2, but also in the mosses themselves and in the later stages of digestion and analysis. Random variation in atomic absorption spectroscopy analysis at or near detection limits can cause errors of 50% or more, which emphasises the need for a sufficient dry weight to digest. There are obviously a number of steps which can be taken to reduce the variability, such as increasing the dry weight and homogenizing the material from many shoots (Table 9) or even many plants. These steps do however increase the amount of material needed and this in turn increases the time required to complete the analysis.

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