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Interspecific differences in Zn, Cd and Pb accumulation by freshwater algae and bryophytes

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Abstract

The relationships between the concentrations of zinc, cadmium and lead in aquatic plants and the concentrations of these metals in the ambient water have been compared for three algae (*Lemanea fluviatilis*, *Cladophora glomerata*, *Stigeoclonium tenue*), one liverwort (*Scapania undulata*) and three mosses (*Amblystegium riparium*, *Fontinalis antipyretica*, *Rhynchostegium riparioides*). The data to establish these relationships are all based on our own studies, some published already, some here for the first time. They come from a wide range of streams and rivers in Belgium, France, Germany, Ireland, Italy and the U.K. There were significant bivariate positive relationships between concentrations of Zn, Cd and Pb in water and plant for all species except Cd and Pb in *Stigeoclonium tenue*. When relationships were compared using datasets with total or filtrable metals in water, most differences were slight. However there were marked differences both between species and between metals. Comparison for the seven species of Zn in the plant when aqueous Zn is 0.01 mg l^{-1}, a concentration at which all seven were found, shows that the four bryophytes had the highest concentrations; however the two green algae had steeper slopes (representing change in concentration in plant in response to change in aqueous concentration). *Lemanea fluviatilis* had a slope closer to that of the bryophytes, but the concentration was about one order of magnitude lower. All seven species were found at a concentration of 0.01 mg l^{-1} Pb, and at this concentration there were almost two orders of magnitude difference between the species which accumulated the most (*Scapania undulata*) and the one which accumulated the least (*Cladophora glomerata*). The steepest slope was however shown by *C. glomerata*.

When multiple stepwise regression was applied, the aqueous metal under consideration was the first variable extracted in only nine of the 21 regressions. However one of the other heavy metals (aqueous or accumulated) was extracted first in all but one of the other regressions, presumably because the occurrences of Zn, Cd and Pb were strongly cross-correlated. The principal non-heavy metal factor extracted for Zn and Cd, but not Pb, was aqueous Ca. The relevance of these results to the use of aquatic plants for monitoring heavy metals is discussed.

Introduction

There are a relatively large number of accounts in the literature relating concentrations of heavy metals (*sensu* Passow *et al.*, 1961) in aquatic plants to concentrations in particular environments. However the concentrations recorded for particular metals sometimes differ markedly.

While it is clear that environmental factors, including the aqueous concentration of the metal itself, are important, it is less clear what genetic differences there are between species with respect to metal accumulation. Evidence that they may be important comes from studies (McLean & Jones, 1975; Burton & Peterson, 1979; Welsh & Denny, 1980; Say *et al.*, 1981) where several species harvested from the same site show different metal concentrations. For instance, Burton and Peterson (1979) found that *Hygrohypnum ochraceum* and *Philonotis fontana* from the same site (although presumably different microhabitats) in R. Ystwyth, Wales, had 780 and 2064 $\mu g\ g^{-1}$ Zn, respectively. It was therefore decided to make a more detailed comparison for a range of algae and bryophytes. Such information is important when selecting which species to use for monitoring heavy metals (Whitton *et al.*, 1981).

In order to make most effective use of metal concentrations in a particular species as a means of monitoring aqueous metal concentrations, the following should be known: relationship between concentration in plant and water; influence of environmental factors on this relationship; rate of loss following an environmental downshift in metal concentration or uptake when there is an upshift. The present study is concerned with the first two features and is based on comparative data for seven species collected from a wide range of field sites. Interpretation of differences between species is based largely on statistical comparisons between results from these studies, although this is helped in some cases by the fact that data have in some cases been collected for several species from the same site at the same time.

Metal accumulation by seven species (three algae and four aquatic bryophytes) is considered here. The information needed for making statistical comparisons is based both on published and previously unpublished studies from our own laboratory (see Methods). It proved impossible to include angiosperms in the comparison, because no study has yet provided a sufficiently detailed dataset. Angiosperms typically occupy a narrower range of habitats than the algae and bryophytes included here, making it difficult to find a wide enough range of metal concentrations from which to sample. In addition there may be complex interactions between the plant and sediments (Denny, 1972; Welsh & Denny, 1979). Possible relationships between the algae and bryophytes dealt with here and sediments have been considered, but as it seems likely that any effects are mostly indirect, they are not included. Say *et al.* (1981) reported significant positive correlations between metal concentrations in plant and water, but not between plant and sediment for *Fontinalis squamosa* and *Rhynchostegium riparioides* individually, although there were significant correlations between plant and sediment for pooled data of a number of species. Use of a larger dataset for *Fontinalis antipyretica* (Say & Whitton, 1983) also showed a significant positive correlation between moss and sediment. The relatively simple morphologies of algae and bryophytes mean that differences in concentrations will reflect differences in the mode of accumulation rather than partitioning of metals between waters and sediments (e.g. Hébrard *et al.*, 1968).

Materials and methods

The data used for statistical comparisons were collected over the period 1975–1982 (Table 1). Reference to a species is by its generic name only in Methods and Results. Publications on three species not previously reported are being prepared and the additional data used for the other species can be made available if required by other researchers. The majority of samples have been collected from the Northern Pennine Orefield in England, but others come from elsewhere in the U.K. and Belgium, France, Germany, Ireland and Italy (Sardinia). Almost all samples have been taken predominantly in late spring and summer from designated 10-m lengths of stream or river termed a reach (Holmes & Whitton, 1981); the exceptions are where a river is wide in comparison with the length of a reach.

As data have been collected over a long period, it is inevitable that minor changes in methodology have taken place. Physico-chemical variables

Table 1. Source of data for statistical comparisons. The value in brackets for *Stigeoclonium* refers to size of dataset using 4 replicates from all but one site (see Methods).

		Reference	Additional samples	Total samples
Rhodophyta	*Lemanea fluviatilis* (L.) Agardh	Harding & Whitton (1981)	26	75
Chlorophyta	*Cladophora glomerata* (L.) Kütz.	unpublished		60
Chlorophyta	*Stigeoclonium tenue* Kütz.	unpublished		27 (105)
Hepaticae	*Scapania undulata* (L.) Dum.	Whitton *et al.* (1982)		52
Musci	*Amblystegium riparium* (Hedw.) Br. Eur.	unpublished		52
Musci	*Fontinalis antipyretica* Hedw.	Say & Whitton (1983)	5	59
Musci	*Rhynchostegium riparioides* (Hedw.) C. Jens	Wehr & Whitton (1983a)		105

were measured *in situ*, but other variables were measured on return to the laboratory. It is possible to make some general comments on collection of samples for metal analysis. 'Total' sample refers to water collected from the stream in 2 l polyethylene beakers and allowed to stand for five minutes to permit larger suspended particles to settle; water was then decanted from the top of the beaker. 'Filtrable' samples were passed through a 0.2 μm Nuclepore filter. All samples were acidified with either Aristar HCl (– 1981) or atomic absorption grade HNO_3 (1982 –) and stored in the dark at 4 °C prior to analysis. Phosphorus (as filtrable reactive phosphate, FRP) was measured by modifications of the molybdate method (Stainton *et al.*, 1977; Mackereth *et al.*, 1978) and chloride by argentometric titration (American Public Health Association, 1981) to 1977 and subsequently with an Orion ion-specific electrode.

For all species except *Stigeoclonium* 2-cm apical tips (1-cm for *Scapania*) were used. They were washed in deionized water in the laboratory, dried at 105 °C and digested in boiling HNO_3 (concentrated or 2 M) in boiling tubes for 30 – 45 minutes, cooled, centrifuged twice to remove detritus and made up to 25 ml in volumetric flasks. Analysis of water and plant digest samples was performed on Perkin-Elmer 403 (to 1982) or 5000 (post 1982) atomic absorption spectrophotometers with graphite furnace attachments for low concentrations of cadmium and lead.

The size of datasets listed in Table 1 in general refers to the number of different sites sampled, but

a few species were sampled more than once atomic absorption spectrophotometers with graphite furnace attachments for low concentrations of cadmium and lead.

The size of datasets listed in Table 1 in general refers to the number of different sites sampled, but

a few species were sampled more than once from the same site if there had been an obvious change in aqueous chemistry. Almost all of the *Stigeoclonium* sites are represented by four replicates (collected on same day from different places within stream reach), enabling data to be considered as a mean of these four samples (Table 1) or, for the multiple regression calculations, as an expanded dataset (n = 105). The number of variables (environmental and metal composition of plant) also differed between studies. In order to produce a 'standard' dataset for statistical comparisons, subsets of each dataset were selected, each containing only those variables present in all datasets. These comprised 13 variables: in water – pH, total Ca, total Mn, total Fe, total Zn, total Cd, total Pb; in plant – Ca, Mn, Fe, Zn, Cd, Pb.

Data were processed with the Northumbrian Universities Multiple Access Computer (NUMAC) using an Amdahl 470/V8 and running under the Michigan Terminal System (MTS). Transformations and most subsequent statistics were performed using subroutines within the Michigan Interactive Data Analysis System (MIDAS: Fox & Guire, 1976). Comparison of regression coefficients was performed using a custom-written Fortran 77 program. This performed a modified form of Analysis of variance

(ANOVA) in which the sum of the deviations around the two individual regressions are compared with the deviation around a single relationship computed from pooled data (Parker, 1979; Mead & Curnow, 1983).

Multivariate statistical techniques were used to identify those factors which account for the variation in the accumulated metal (as the dependant variable). An iterative forward-selection multiple stepwise regression was used which added new variables to the equation as long as they attained a predetermined level of significance ($p < 0.05$ for this study). If at a later stage this fell below $p > 0.10$ it was removed from the equation. (See Draper and Smith, 1981, for a fuller explanation.)

Results from such studies are not proof of effects (Draper & Smith, 1981); however, when models constructed by this approach of factors affecting accumulation by *Rhynchostegium riparioides* (Wehr & Whitton, 1983) were tested in a subsequent experimental study (Wehr *et al.*, in press) there was broad agreement between the two approaches.

Results

Bivariate relationships

There were significant bivariate positive relationships between concentrations of Zn, Cd and Pb in water and plant for all species except Cd and Pb in *Stigeoclonium* and Cd in *Fontinalis* (Figs 1–3). When relationships were compared using datasets with total or filtrable metals in water (see Methods), nine of the fifteen comparisons were not significant; however two comparisons for each of *Lemanea*, *Scapania* and *Amblystegium* were (Table 2). There were also marked differences between species (Table 3) and between metals (Table 4). For instance, four of the six species for which data were available for all metals showed steeper regression coefficients (slopes) for Pb than for Zn and Cd. The lowermost concentrations of metals shown for each species depend in part on the analytical detection limits used in any particular study. On the other hand the upper limit indicates the highest metal concentration recorded for a species and presumably gives some indication of the ability of that species to tolerate the metal: compare, for instance the Zn ranges for *Fontinalis* and *Scapania* (Fig. 1). In this study *Cladophora* was found at aqueous Zn concentrations up to 0.167 mg l^{-1}; one other site, on the R. Gueule in Belgium (1002–50) extended this range (see dotted lines on Figs 1 & 3) to 0.92 mg l^{-1} and 0.183 mg l^{-1} Pb. The effects of these concentrations are perhaps ameliorated by high concentrations of aqueous Ca (59.2 mg l^{-1}) and FRP (0.546 mg l^{-1}).

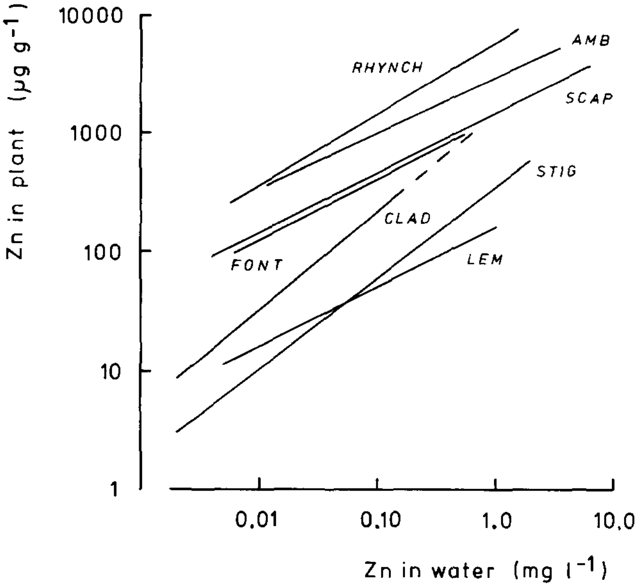

Fig. 1

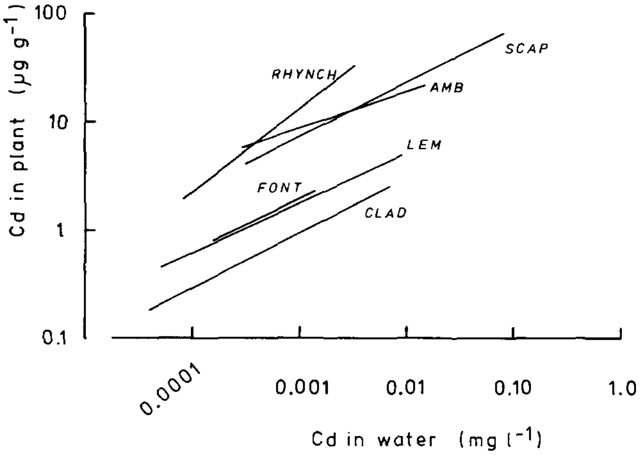

Fig. 2

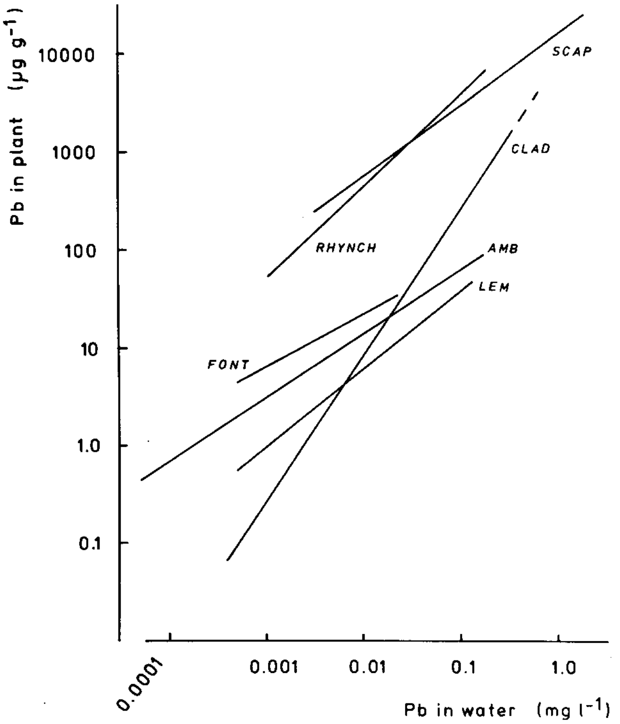

Fig. 3

Figs 1–3. Influence of aqueous metals concentration on metal concentration of plants for Zn, Cd and Pb. LEM, *Lemanea fluviatilis*; CLAD, *Cladophora glomerata*; STIG, *Stigeoclonium tenue*; SCAP, *Scapania undulata*; AMB, *Amblystegium riparium*, FONT, *Fontinalis antipyretica*; RHYNCH, *Rhynchostegium riparioides*. Dashed line = extension to include R. Gueule in Belgium (not included in statistics). Note for *Stigeoclonium tenue*, data for Zn only.

Zn. Comparison for the seven species of Zn in the plant when aqueous Zn is 0.01 mg l^{-1}, a concentration at which all were found (Fig. 1), shows that the four bryophytes had the highest concentrations; however the two green algae had steeper slopes (representing change in concentration in plant in response to change in aqueous concentration). *Lemanea* had a slope closer to that of the bryophytes, but the concentration was about one order of magnitude lower. The extent to which differences between slopes are significant is shown in Table 3.

Table 2. Comparison of regression coefficients obtained using total or filtrable water in regressions relating to metal in plant and metal in water. Only significant regressions included. (No data with filtrable water available for *Fontinalis.*) Data given as variance ratio F, together with probability. * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$.

	Zn	Cd	Pb
Lemanea	12.316***	–	23.04**
Cladophora	0.111	0.018	0.239
Stigeoclonium	2.03	–	–
Scapania	1.158	8.362**	6.585*
Amblystegium	0.070	8.124*	9.097**
Rhynchostegium	0.041	0.022	0.026

Cd. All seven species were found at a concentration of 0.001 mg l^{-1} Cd and at this concentration three bryophytes (*Scapania, Amblystegium, Rhynchostegium*) contained considerably more Cd than the other species. There was less difference between the slopes for the various species than for Zn or Pb.

Pb. All seven species were found at a concentration of 0.01 mg l^{-1} Pb and at this concentration there was almost two orders of magnitude difference between the species which accumulated the most (*Scapania*) and the one which accumulated the least (*Cladophora*). The steepest slope was shown by *Cladophora.*

Slopes of different metals by a particular species were also compared (Table 4). In the case of *Rhynchostegium*, the slope for Pb was significantly steeper than that for Zn, but neither the difference between Zn and Cd nor Cd and Pb were significant.

The 'goodness-of-fit' of these bivariate relationships was measured using the coefficient of determination, r^2 (Table 5). Values obtained using total or filtrable metals in water were always similar. The range of r^2 values was least for Zn (0.52 – 0.74), intermediate for Cd (0.09 – 0.43) and most for Pb (0.14 – 0.65). Although the regressions were all significant (based on ANOVA), the goodness-of-fit ranged from 0.65 (Zn in *Lemanea*) to less than 0.18 (Pb in *Amblystegium*).

Table 3. Comparison of regression coefficients relating to metal in plant and metal in water for all species; only significant regressions included. Data give as variance ratio F, together with probability, * < 0.05, ** < 0.01, *** < 0.001

Zn							
Lemanea	–						
Cladophora	7.11**	–					
Stigeoclonium	2.214	0.001	–				
Scapania	0.015	5.274*	3.690	–			
Amblystegium	0.024	5.77*	2.052	0.0003	–		
Fontinalis	0.096	5.064*	2.176	0.238	0.166	–	
Rhychostegium	1.30	3.54	2.702	1.714	1.45	0.651	–
	Lemanea	*Cladophora*	*Stigeoclonium*	*Scapania*	*Amblystegium*	*Fontinalis*	*Rhynchostegium*
Cd							
Lemanea	–						
Cladophora	0.438	–					
Scapania	0.234	0.084	–				
Amblystegium	0.009	0.583	0.375	–			
Fontinalis	1.317	4.583*	0.094	1.641	–		
Rhychostegium	5.719*	2.168	7.655**	7.655**	1.051	–	
	Lemanea	*Cladophora*	*Scapania*	*Amblystegium*	*Fontinalis*	*Rhynchostegium*	
Pb							
Lemanea	–						
Cladophora	4.408*	–					
Scapania	0.089	5.238*	–				
Amblystegium	0.204	1.028	0.301	–			
Fontinalis	0.641	9.503*	0.791	1.499	–		
Rhychostegium	2.615*	5.238*	3.404	9.499**	6.796*	–	
	Lemanea	*Cladophora*	*Scapania*	*Amblystegium*	*Fontinalis*	*Rhynchostegium*	

Table 4. Regression coefficients (in order of decreasing magnitude) relating accumulated and aqueous concentrations of metals; only significant regressions included. Only the difference between Pb and Zn in *Scapania* is significant

Lemanea	Pb (0.695) > Zn (0.510) > Cd (0.405)
Cladophora	Pb (1.179) > Zn (0.772) > Cd (0.536)
Scapania	Pb (0.711) $\overset{*}{>}$ Zn (0.527) > Cd (0.487)
Amblystegium	Zn (0.496) > Cd (0.389) > Pb (0.236)
Fontinalis	Zn (0.536) > Pb (0.524) > Cd (0.450)
Rhynchostegium	Pb (0.934) > Cd (0.759) > Zn (0.597)

Table 5. Values for coefficient of determiation (r^2) for bivariate relationship between concentrations of metal in plant and metal in water. Where possible (from dataset), values given for both total (T) and fitrable (F) water.

	Zn		Cd		Pb	
	T	F	T	F	T	F
Lemanea	0.66	0.66	0.18		0.36	
Cladophora	0.61	0.59	0.25	0.22	0.48	0.36
Stigeoclonium	0.74	0.63				
Amblystegium	0.59	0.53	0.28	0.37	0.64	0.65
Fontinalis	0.55	0.52	0.26	0.39	0.18	0.19
Rhynchostegium	0.59		0.09		0.14	
	0.65	0.64	0.42	0.43	0.56	0.49

Multivariate relationships

The goodness-of-fit of bivariate relationships may be improved by the stepwise inclusion of additional variables into the regression equation. However, this does not necessarily improve the precision of the estimate because each additional variable is accompanied by its own error term. Before this was examined a preliminary analysis was performed to look at the improvement in predictions brought about by the inclusion of variables, which have no significant correlation with the metal of interest, either in water or in plant. Na and Cl were chosen as examples of elements which are generally considered to be 'conservative' in their behaviour in freshwater and which are not usually implicated as factors influencing metal accumulation or loss. Three of the datasets included analyses of Na and Cl and one other included Na. The maximum effect observed when Na or Cl was added to a significant regression was a 0.07 improvement in r^2 (Table 6), although the mean 'improvement' was much lower (Zn: 0.02; Cd: 0.03; Pb: 0.01; based on data for Na and Cl pooled). This baseline gives some indication of the importance to be placed on different values for r^2.

Multiple stepwise regression analyses were then applied to large groups of variables. The examples given in Table 7 all made use of an identical set of variables (see Methods). The aqueous metal under consideration was the first variable extracted in only nine of the 21 regressions. One of the other heavy metals (aqueous or accumulated) was extracted first in all but one of the other regressions, presumably because the

Table 6. Effect of incorporating the biologically 'conservative' elements Na and Cl into bivariate regression equations relating to metal in plant and metal in water. Values as coefficient of determination r^2.

Metal	Species	r^2		
		– element	+ element	Difference
Zn	*Cladophora*	0.59	Na 0.61	0.02
			Cl 0.60	0.01
	Stigeoclonium	0.63	Na 0.68	0.05
			Cl 0.64	0.01
	Amblystegium	0.55	Na 0.57	0.02
	Rhynchostegium		Na 0.64	0.00
			Cl 0.65	0.01
Cd	*Cladophora*	0.22	Na 0.24	0.03
			Cl 0.29	0.07
	Amblystegium	0.50	Na 0.55	0.05
	Rhychostegium	0.43	Na 0.43	0.00
			Cl 0.43	0.00
Pb	*Cladophora*	0.37	Na 0.37	0.00
			Cl 0.37	0.00
	Amblystegium	0.20	Na 0.22	0.02
	Rhychostegium	0.49	Na 0.50	0.01
			Cl 0.50	0.01

Table 7. Variables extracted in multiple stepwise regressions (with influence) using A) Cd and C) Pb in plants as dependent variables. Independent variables are those common to all species and metals. In two instances the placing of a variable (underlined) was changed at a late step (see Methods).

A) Zn species	Size dataset	Steps							r^2	
		1	2	3	4	5	6	7	Initial	Final
Lemanea	44	Zn_{aq} (+)	Pb_{aq} (−)	Pb_{pl} (+)	Ca_{aq} (−)	Ca_{pl} (+)	Mn_{pl} (−)		0.68	0.89
Cladophora	66	Zn_{aq} (+)	Mn_{pl} (+)	Ca_{aq} (+)					0.68	0.89
Stigeoclonium	65	Zn_{aq} (+)	Ca_{aq} (−)	Cd_{aq} (+)	Mn_{pl} (−)	Mn_{aq} (−)	$\underline{Zn_{aq}}$ (+)		0.35	0.61
Scapania	44	Cd_{pl} (+)	Pb_{pl} (+)	PH (+)					0.59	0.86
Amblystegium	20	Zn_{aq} (+)	Cd_{pl} (+)						0.59	0.67
Fontinalis	54	Zn_{aq} (+)	Pb_{pl} (+)	Mn_{pl} (+)	Cd_{pl} (+)	Fe_{aq} (−)			0.59	0.79
Rhynchostegium	105	Zn_{aq} (+)	Cd_{pl} (+)	Mn_{pl} (+)	Ca_{aq} (−)	Ca_{pl} (+)	Fe_{aq} (−)		0.65	0.88
Lemanea	44	Zn_{pl} (+)	Fe_{aq} (+)						0.41	0.52
Cladophora	60	Cd_{aq} (+)	Ca_{pl} (+)	Pb_{pl} (−)					0.25	0.37
Stigeoclonium	65	Zn_{aq} (+)	Fe_{pl} (−)	Pb_{pl} (+)					0.18	0.41
Scapania	44	Zn_{pl} (+)	Ca_{aq} (+)	Fe_{pl} (−)					0.59	0.72
Amblystegium	20	Cd_{aq} (+)	Zn_{pl} (+)						0.46	0.63
Fontinalis	54	Zn_{pl} (+)	Mn_{pl} (−)	Ph (+)					0.31	0.48
Rhynchostegium	105	Zn_{pl} (+)	Pb_{pl} (+)	Cd_{aq} (+)	Zn_{aq} (−)				0.30	0.76
Lemanea	77	Zn_{aq} (+)	Fe_{pl} (+)	Pb_{aq} (+)	Mn_{aq} (−)	Mn_{pl} (−)	Fe_{aq} (−)	$\underline{Mn_{aq}}$ (−)	0.43	0.79
Cladophora	60	Fe_{pl} (+)	Pb_{aq} (+)	Zb_{pl} (+)					0.60	0.83
Stigeoclonium	65	Zn_{pl} (+)	Cd_{pl} (+)						0.17	0.27
Scapania	77	Zn_{aq} (+)	Fe_{pl} (+)	Pb_{aq} (+)	Fe_{aq} (−)	Cd_{pl} (+)			0.67	0.91
Amblystegium	20	Cd_{aq} (+)	Zn_{pl} (+)	pH (−)					0.55	0.72
Fontinalis	54	Zn_{pl} (+)	Pb_{aq} (+)						0.32	0.38
Rhynchostegium	105	Pb_{aq} (+)	Cd_{pl} (+)	Fe_{aq} (−)	Fe_{pl} (+)	Mn_{aq} (−)	Zn_{pl} (+)	Ca_{pl} (+)	0.56	0.82

occurrence of Zn, Cd and Pb was strongly correlated (see Methods and publications on individual species). The principal non-heavy metal factor extracted for Zn and Cd, but not Pb, was aqueous Ca.

Zn. Zn_{aq} was the first variable extracted from all datasets except that for *Scapania*, although it was removed at a later stage from *Stigeoclonium*. Regression equations included from two to six variables and accounted for 0.61 – 0.89 of the variability, a mean improvement of 0.20 (S.D. = 0.07) over bivariate regressions.

Cd. Cd_{aq} was the first variable extracted from only two of the datasets (*Cladophora*, *Amblystegium*). For the others, Zn, either in water (*Stigeoclonium*) or plant (*Lemanea*, *Scapania*, *Fontinalis*, *Rhynchostegium*) was the first variable extracted. Up to three other variables were extracted and the maximum value of r^2 was 0.76. This represents a mean improvement of 0.20 (S.D. = ± 0.12) over bivariate regressions. As Cd_{aq} was not selected in the first step for *Stigeoclonium* and *Fontinalis*, the values of r^2 at step 1 are higher than for the bivariate relationships described above.

Pb. Pb_{aq} was the first variable extracted only for *Rhynchostegium*. Other heavy metals, either in the water or the plant, filled this position for all except *Cladophora*, for which Fe_{pl} was extracted first. Pb_{aq} was extracted at step 2 or 3 for four other species, but did not feature in the *Stigeoclonium* of *Amblystegium* datasets. Up to seven steps were performed; these all added variables to the regression equation except step 7 with *Lemanea*, which removed Mn_{aq}. The final equations accounted for between 0.27 (*Stigeoclonium*) and 0.91 (*Scapania*) of the variability, a mean improvement of 0.20 (S.D. = 0.10) over bivariate equations.

A further set of analyses for five species (*Lemanea*, *Cladophora*, *Stigeoclonium*, *Fontinalis*, *Rhynchostegium*) added filtrable reactive phosphate (FRP) as an extra variable. In only a few cases was FRP extracted as step. In the case of Zn, FRP was extracted only in the *Cladophora* dataset, as the fourth and final variable. For Cd, FRP was the fourth and final variable extracted from the *Fontinalis* dataset. For Pb, FRP was extracted from the *Rhynchostegium* dataset at step 3 but subsequently removed at step 7.

The r^2 value gives an indication only of the precision of the results and not the accuracy. In order to assess this, scattergrams of the values predicted by multivariate equations and actual concentrations were checked for differences in slopes and intercepts. No marked differences were shown and so no further analyses were performed.

Discussion

Examples of obvious differences between species were observed with all three metals. As the study was based on statistical comparisons, some comments on methods and interpretation of results are needed. Concentrations have been expressed as $mg\,l^{-1}$ and $\mu g\,g^{-1}$, since these units provide data for monitoring purposes which are easy to interpret. Use of units such as mM or $\mu mol\,g^{-1}$ would have no effect on statistics which assess relative contributions to variability. A degree of biological intuition is required to interpret the effects. In particular, there are correlations between several of the variables (e.g. aqueous Zn v aqueous Cd). Although use of correlated independent variables is justified (Mead, 1971), this does make interpretation of the results more difficult. Is it a real biological effect or just a statistical artefact?

The bivariate plots showing the accumulation of Zn, Cd and Pb give a clear visual indication of how accumulation of these metals varies between species. For Zn, the species fall into three groups:

i. *Lemanea fluviatilis* – slope similar to bryophytes, but lower intercept;
ii. the two green algae – lower absolute concentrations, but steeper slopes;
iii. bryophytes – with high concentrations.

It is difficult to compare the absolute concentrations of Zn and Cd accumulated from a particular metal concentration, because there is no one concentration at which all species occur for both metals. If the Cd slopes for *Amblystegium riparium*, *Fontinalis antipyretica* and *Rhynchostegium riparioides* (Fig. 2) are extended slightly to 0.004 mg l^{-1}, however, the accumulation ratio is less in every case for Cd. Another obvious difference between Cd and Zn is the much lower Cd concentration in *Fontinalis antipyretica* than the three other bryophytes. In view of the similarities in environmental chemistry of Zn and Cd (Hen, 1972), it is difficult to suggest explanations for these difference.

In the case of Pb, 0.01 mg l^{-1} metal provides a concentration for which comparison may be made with Zn. The concentrations of Pb in the plant are higher with *Rhynchostegium riparioides* and *Scapania undulata*, but much lower with *Amblystegium riparium* and *Fontinalis antipyretica*. The most striking feature for Pb is the slope for *Cladophora glomerata*, which is much steeper than for any other species or element (Tables 3, 4).

Some of these results may be interpreted in the light of theories on the mechanisms of metal accumulation by cryptogams. There is a substantial literature which points to a considerable cation exchange capacity of bryophytes (Clymo, 1963; Brown & Beckett, 1985) and we have recently suggested that the low concentration of Ca in *Lemanea fluviatilis* might result from a low concentration of cation-exchange sites (Kelly & Whitton, 1987). Differences in the concentration of such sites would lead to differences in the vertical position (intercept) of the regression line.

The cell wall is not the only site of metal accumulation in bryophytes (Brown & Beckett, 1985; Wehr *et al.*, 1987). Such studies have separated accumulation into different compartments, each with different kinetics and capacities. The steeper slopes for *Cladophora glomerata* and *Stigeoclonium tenue* may reflect different contributions by these various compartments.

All the species studied here are among the ten recommended for analysis when monitoring heavy metal pollution in U.K. waters (Whitton *et al.*, 1981). The comparisons given in this paper show that *Rhynchostegium riparioides* and *Scapania undulata* are likely to be the species most useful for monitoring purposes under conditions where steady state conditions of pollution by Zn, Cd and Pb. The high accumulation ratios shown with these species mean that less plant material is required and that analyses can be carried out further above detection limits. On the other hand the steep slopes found for *Cladophora glomerata* suggest that this species may be useful where it is important to distinguish between two different sites, especially where the difference in concentration of pollutant metal is suspected to be relatively small. This would also apply when long-term changes at a site are being monitored. The datasets used here do not provide any guidance for monitoring short-term changes, because information on rates of uptake and loss would be needed. However the much more rapid growth rates typically shown by the two green algae suggest that these may be especially useful for this purpose. The other four species in the present paper are all potentially useful in particular environments, where the other three may not occur. For instance, *Lemanea fluviatilis* is the species most likely to be found in fast-flowing waters in winter and spring, *Amblystegium riparium* in highly polluted waters and *Fontinalis antipyretica* in mesotrophic lakes.

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