METAL ACCUMULATION AND PIGMENT STRESS OF AQUATIC BRYOPHYTES FROM THE RIVER EUME, GALICIA (NW SPAIN)

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ABSTRACT

A study is made of metal accumulation (Cu, Cd, Zn, Pb, Ni, Mn and Fe) and pigment stress caused in the following bryophytes: *Fontinalis antipyretica* Hedw., *Rhynchostegium riparioides* (Hedw.) Card., *Fissidens polyphyllus* Wils. and *Scapania undulata* (L.) Dum., collected from the river Eume, Galicia (NW Spain). This river bears a gross metal pollution due to an open cast mine of a termal power station set up on its basin.

S. *undulata* and *F. polyphyllus* were the species with the maximum and minimum accumulative efficiency respectively. Ni showed an exceptional mean value, higher than any published value. Correlations between metal concentrations in water-bryophytes were very low being significant only for Zn and Mn.

The damaging effects of heavy metals on bryophytes can be observed in the high correlations found between pigment stress (ratio D665/D665a) and metal concentration. The evaluation of water quality by means of the calculation of contamination factors is also discussed.

INTRODUCTION

The particular morphology and physiology of aquatic bryophytes allow them to be hyperaccumulators of heavy metals in water. This capacity has led to numerous studies (MOUVET, 1979, 1980 and 1984a; EMPAIN *et al.,* 1980; SAY *et al.,* 1981; WEHR *et al.,* 1981; WHITTON *et al.,* 1981; JONES *et al.*, 1985; MOUVET *et al.*, 1986; ANDRE & LASCOMBE, 1987; MASON & MACDONALD, 1988). Metal relationship bryophyte-water has also been widely studied (WHITTON *et al.,* 1982; SAY & WHITTON, 1983; WEHR & WHITTON, 1983; EMPAIN, 1988) as well as the relation of accumulation efficiency among different species (JONES, 1985; KELLY & WHITTON, 1989). Many of these papers have shown that aquatic bryophytes have higher concentration factors for metals than sediments and phanerogams (DIETZ, 1973).

The studies on the relation bryophyte-water have been performed mainly with total and filtrable metal, being rare the studies about the influence of the chemical speciation of metals on the bioaccumulation (MOUVET, 1984b). Heavy metal pollution specially damages plant leaves. Chloroplasts and mitochondria are the most damaged organelles, which involves the alteration of the photosynthetic pigments and the decrease of chlorophyll biosynthesis (BARCELO & POSCHENRIEDER, 1989 and 1990). The consequences of metal pollution on the biology of aquatic bryophytes, even knowing their importance as primary producers in the aquatic ecosystems, have not been as studied as their accumulation efficiency (MCCLEAN & JONES, 1975; EMPAIN, 1977; SATAKE *et al.*, 1989).

The objective of this study is to compare the efficiency of metal amplification among different species, and the relationship between metal concentration in bryophytes and different chemical forms of metal in water. At the same time we try to analyse how plant physiology is affected by metal contamination. This analysis is based on the characterization of pigment responses to pollution. Finally, we try to evaluate the degree of metal pollution in the study area, by means of metal concentrations in bryophytes.

MATERIALS AND METHODS

This study was made in the basin of river Eume (Galicia NW Spain). This area bears an important mining and industrial activity due to a termal power station; the river does not show a serious organic pollution. Samples were taken from twelve stations; four of them in undisturbed tributaries. The remaining stations were distributed along the stream, four sites being down stream from the power station (Fig. 1).

(Nesslerization method), FRP (filtrable reactive phosphate, by Ascorbic Acid method), COD (Consumption of $KMnO_4$, 10 min), Cu, Zn, Cd and Pb (polarographyc method, separating in each sample the fractions: free in solution, complexed in solution, total in solution, particulate metal and total metal) and

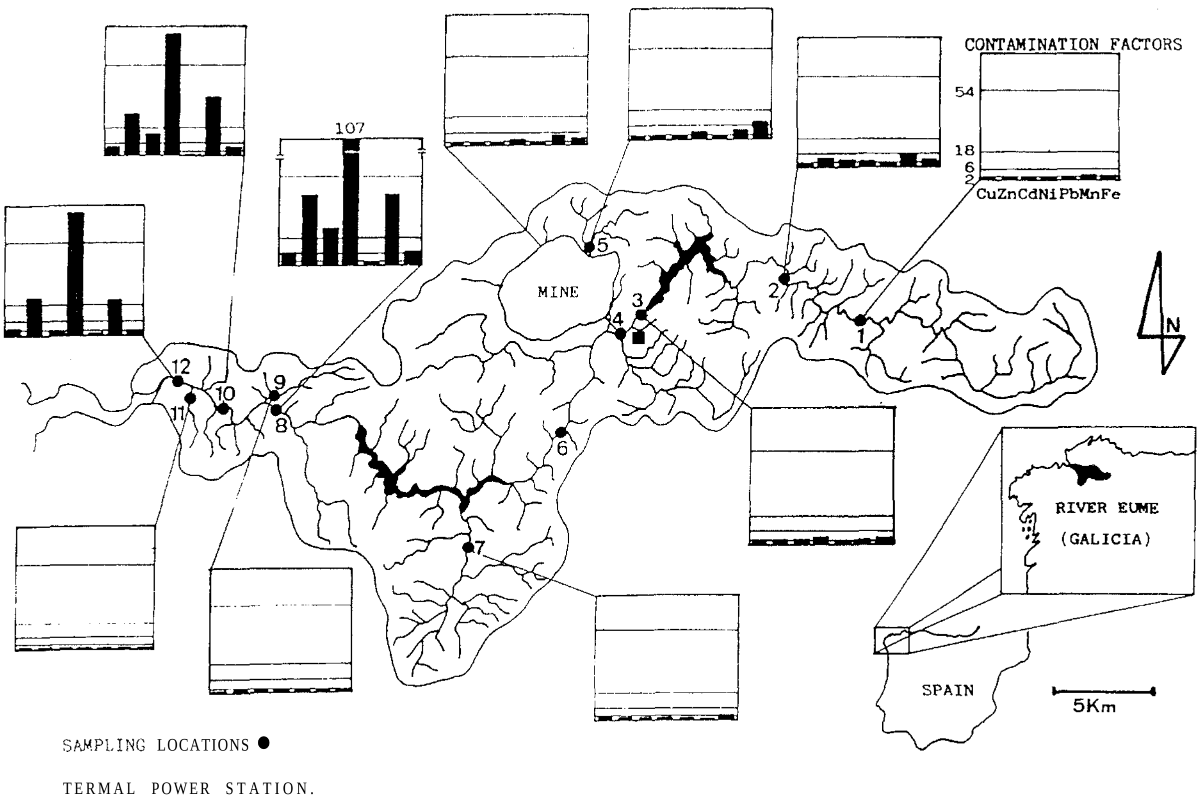

FIGURE 1. Mean contamination factors for each metal in the aquatic bryophytes in river Eume catchment.

Wherever possible samples were collected of the following species: *F. antipyretica* Hedw., *R. riparioides* (Hedw.) Card., *F. polyphyllus* Wils. and *S. undulata* (L.) Dum. Samples were taken from various locations within each sampling station (comprising a 100 m stretch of river), in areas of rapid flow, below the low water level at depths of less than 60 cm.

Prior to briophyte sampling four water samples were taken (once a week) in the stations: 1, 3, 5, 6, 7, 8, 10, 11 y 12 (Fig. 1) for physical and chemical analysis by conventional methods (Standard Methods, 1985). Water samples were collected in 2 l polyethylene beakers. Temperature, pH, conductivity and oxygen were determined in situ; Cl^- (Argentometric method), SO_4^{2-} (Turbidimetric method), Ca (EDTA Titrimetric method), NO_3^- (Ultraviolet Spectrophotometric method), NO_2^- (Sulfanilic acid + NED, Spectrophotometric method), NH_4^+ filtrable (Teflon filter) Mn (Formaldoxime method) and Fe (Phenanthroline method) were determined in the laboratory within 48 hours, stored in the dark at 4 °C.

Bryophyte samples were washed several times in stream water to remove attached particles. They were stored in refrigerated boxes (5±2 "C) and brought back to the laboratory where the terminal 2 cm. tips were removed to give a fresh weight of aproximately 10 g. The tips were thoroughly washed with deionized water for two hours (20±2 "C) dried to constant weight at 45±5 °C and then ground in an agate mill.

Total digestion of organic matter and extraction of metals from each sample (250 - 300 mg) was carried out using nitric acid (10 ml supra-pur quality) in Teflon vessels with pressure valves placed in a microwave oven. The mineralization process comprised predigestion for 30 min at atmospheric pressure followed by 2 min 30 s at 750 Watts and 20 min at 300 Watts; samples were then cooled and brought back to atmospheric pressure. This process was repeated three times. Finally, the extract was made up to 50 ml with de-ionized water and purified by filtration (0.45 μM Teflon membrane Milipore filters).

Determination of metal concentration (three replicates per moss sample) was done using flame atomic absorption spectrophotometry (Perkin Elmer 2100). Metals analysed and their detection limits (pg g') were: cadmium (0.008), zinc, copper, iron, nickel and manganese (0.01) and lead (0.02). As a control for the whole process (from extraction to determination) samples of certified reference material (BCR Nº61: *Rhynchostegium riparioides)* were also analyzed. The recovery was higher than 95% for Cu, 'Ni, Pb and Zn; and higher than 90% for Fe and Mn.

The determination of the physiological stress degree in the plant was carried out by studying the pigment changes generated in the different sFies along the river. For pigment analysis the samples were washed and tips pigments were extracted with 90% acetone during one hour at 20±2 °C in darkness. The homogenates obtained were centrifuged at 1500 rpm for three minutes. Then, after the spectrophotometric reading of absorbance (Milton Roy Spectronic 3000 Array), we calculated the following pigments and pigment ratios: Chlorophyll a (VOLLENWEIDER, 1974), Chlorophyll b (LORENZEN, 1967), Chlorophyll a/b, D430/D665 (absorbance at 430 nm/absorbance at 665 nm) and D665/D665a (absorbance at 665 nm/absorbance at 665 nm after the addition of 30 p1 of 1 N HCl to 3 ml of pigment extract) (MARGALEF, 1983; MOUVET, 1984; PEÑUELAS, 1985; MARTÍNEZ & SÁNCHEZ, 1987; ZAPATA, 1988). Pigment contents are referred to plant dry weight.

RESULTS

Metal concentration (relation water-bryophytes).

Physico-chemical characteristics of water are summarized in Table 1. Table 2 shows metal concentrations in bryophyte apical tips. F. *antipyretica* had the highest concentrations for all metals with the exception of Pb and Fe. Both metals got the highest values in *S. undulata*. This latter was the only species studied that disappeared from the stations below the lignite mine.

TABLE 1. Physicochemical characteristics of water in the sampling sites. FRP: filtrable reactive phosphate, FS: free in solution, TS: total in solution, P: particulate, SD: standard deviation, X: mean. Number of samples= 9.

Variable	Unit	Min.	Max.	X	SD
pH	6.0	7.1	6.7	0.3	
Conductivity	$\mu S\ cm^{-1}$	43	158	98	52
Ca	$mg l^{-1}$	1.9	17.6	8.3	6.8
O_2	%Sat	99	104	101	1.5
COD	$mg l^{-1}$	1.3	3.5	2.2	0.7
NO_3^-	$mg l^{-1}$	0.9	2.3	1.4	0.4
NO_2^{2-}	$mg l^{-1}$	0.004	0.016	0.01	0.004
NH_4^+	$mg l^{-1}$	0.014	0.111	0.053	0.04
FRP	$mg l^{-1}$	0.002	0.021	0.016	0.006
Cl^-	$mg l^{-1}$	10.4	15.3	13.0	1.8
SO_4^{2-}	$mg l^{-1}$	1.6	42.6	19.9	18.8
Zn FS	$\mu g l^{-1}$	0.08	4.47	1.69	2.03
TS	$\mu g l^{-1}$	0.88	6.23	3.44	2.18
P	$\mu g l^{-1}$	0.04	3.51	1.34	1.23
Cd TS	$\mu g l^{-1}$	0.028	00.69	0.028	0.019
P	$\mu g l^{-1}$	0.070	0.298	0.183	0.077
Pb FS	$\mu g l^{-1}$	0.10	30.40	8.20	12.12
TS	$\mu g l^{-1}$	1.13	31.57	9.58	12.13
P	$\mu g l^{-1}$	0.63	26.23	13.11	12.08
Cu FS	$\mu g l^{-1}$	0.31	1.46	0.67	0.42
TS	$\mu g l^{-1}$	0.67	1.56	1.00	0.33
P	$\mu g l^{-1}$	0.03	0.26	0.10	0.09
Mn TS	$\mu g l^{-1}$	38	201	111	66
Fe TS	$\mu g l^{-1}$	3	184	68	77

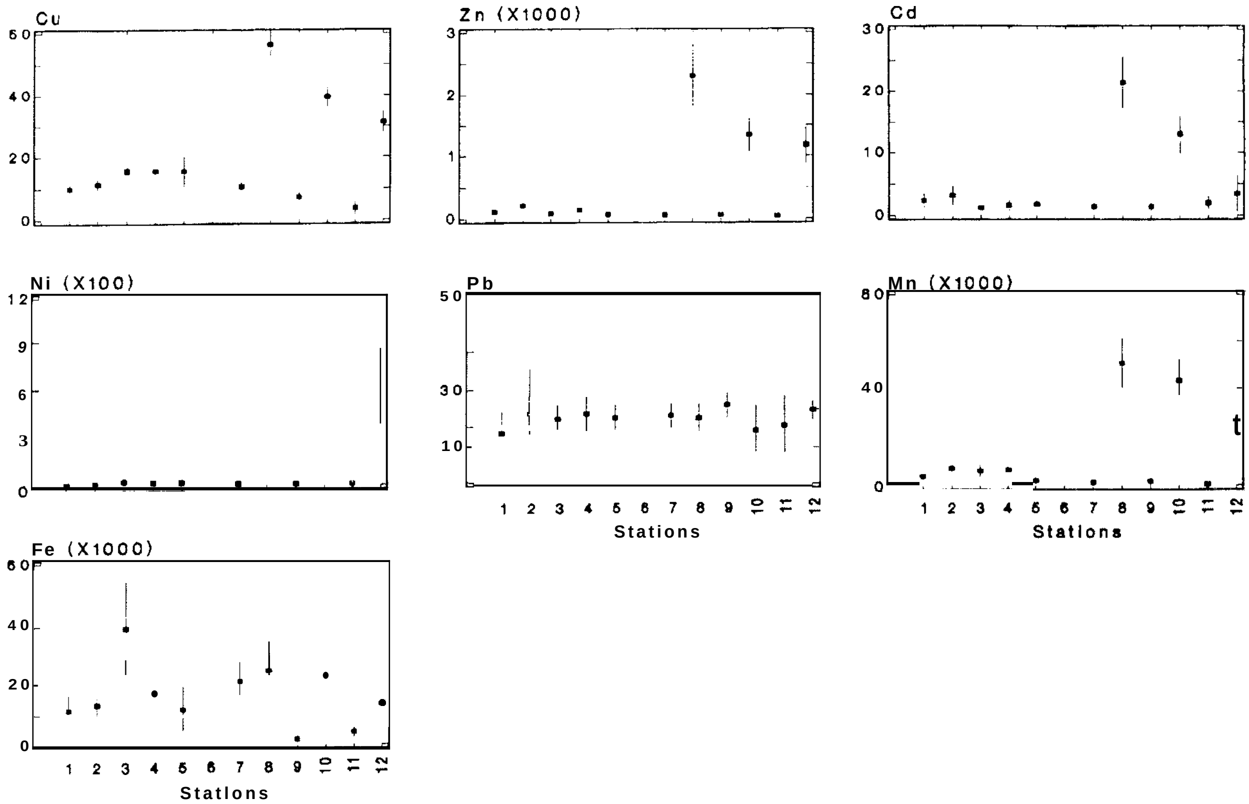

FIGURE 2. Development of mean metal concentrations (μg g-l) on the pooled bryophytes apical tips and confidence intervals (95%) in river Eume.

Mean metal concentrations in shoot tips and their confidence intervals (95%) along the river are given in Fig. 2. Only Pb values remain regular, whereas there is a significant increase of the other metals concentrations at the stations 8, 10 and 12, placed below the mine. It is significant the absence of any bryophyte at station 6 which is placed just below the mine.

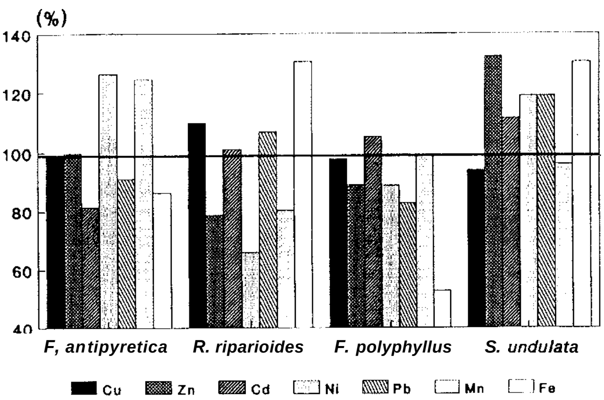

FIGURE 3. Mean interspecific accumulation or relationship between metal concentration in a species aid mean concentration of that metal in all the species (%).

To analyse the accumulation capacity of the different species, we studied the interspecific mean accumulation or relation between metal concentration in one species and the average concentration of that metal in all the species (%). The maximun concentrations of all metals were found in samples of S. *undulata,* except in the cas of Cu and Mn (below 100%), whereas the lowest values for all metals were found in F. *polyphyllus* samples exceeding the average only for Cd (Fig. 2). The relationship between the different forms of metal in water and metal in plant are given in Table 3. Correlations were significant only for Zn and Mn.

Pigment stress (relation stress-metal concentration)

Table 4 shows the values of chorophyll concentrations and pigment ratios of each species studied. F. *antipyretica* presented the largest ranges of variation to any ratio, whereas S. *undulata* presented the lowest ranges. The measurement of the ratio D665/D665a is independent from the extraction errors and pigment predicted equations are not needed, so that this ratio is the most suitable to assess the stress of aquatic bryophytes (LOPEZ

TABLE 3. Main Pearson correlation coefficients (r) among metal concentrations bryophytes-water (FS: free in solution, C: complexed, TS: total in solution, P: particulated, T: total). $^{*}p < 0.05$.

Element		*Fontinalis antipyretica*	*Rhynchostegium riparioides*	*Fissidens polyphyllus*	*Scapania undulata*
Zn	FS	0.87*	0.87*	0.82	0.87*
	C	0.45	0.46	0.45	0.46
	TS	0.88*	0.89*	0.84*	0.88*
	P	0.47	0.46	0.48	0.47
	T	0.94*	0.94*	0.91*	0.94*
Cu	P	0.40	0.37	0.40	0.36
Pb	FS	—	—		0.58
	C	0.83			0.66
	TS	0.20		—	0.67
	P			0.41	—
	T	0.14	—	0.62	—
Mn	TS	0.84*	0.84*	0.84*	0.87*
Fe	TS	0.33	0.30	0.84*	0.39

TABLE 4. Basic statistics of pigment ratios studied in the different species. X: mean, SD: standard deviation

Index		*Fontinalis antipyretica*	*Rhynchostegium riparioides*	*Fissidens polyphyllus*	*Scapania undulata*
D665/D665a	X	1.59	1.63	1.62	1.66
	Min	1.51	1.55	1.57	1.63
	Max	1.67	1.67	1.69	1.69
	SD	0.06	0.05	0.04	0.02
D430/D665	X	1.97	1.96	2.04	1.97
	Min	1.82	1.71	1.95	1.93
	Max	2.03	2.08	2.09	2.02
	SD	0.06	0.11	0.04	0.04
Cl*a*/Cl*b*	X	2.85	3.30	3.28	3.59
	Min	1.70	2.21	2.96	3.22
	Max	3.42	4.19	3.53	4.18
	SD	0.54	0.63	0.18	0.29

TABLE 5. Pearson correlation coefficient (r) between the ratio D665/D665a and metal concentration in aquatic bryophytes. $^{*}p<0.05$; $^{**}p<0.01$

	Fontinalis antipyretica	*Rhynchostegium riparioides*	*Fissidens polyphyllus*	*Scapania undulata*
Cu	-0.82**	*0.66	-0.86**	-0.34
Zn	-0.80**	-0.72*	-0.82**	-0.42
Cd	0.67	-0.63	-0.83**	-0.14
Ni	-0.83**	-0.71	-0.75*	-0.22
Pb	-0.12	-0.34	0.27	-0.49
Mn	-0.82**	-0.73*	-0.86**	-0.74
Fe	-0.34	-0.63	-0.89**	-0.74

& CARBALLEIRA, 1990). Table 5 shows the correlation coefficients between the ratio D665/D665a and the metal concentration in each species, observing significant correlations in *F. antipyretica* and *F. polyphyllus* for Cu, Zn, Cd, Ni and Mn.

Evaluation of metal pollution

Contamination factors were calculated (ANDRE & LASCOMBE, 1987) as a multiplication factors in relation to background values. Reference values were extracted from the stations with the lowest metal pollution levels (Table 6). If we compare these values with those reported by MOUVET et al. (1986), we observe that they are the same for Cd and Pb; lower for Cu, Zn and Ni and higher for Fe and Mn. On the other hand if we compare the same values with those reported by LOPEZ *et* al. (1990) for Galician rivers (NW Spain) we observe that they are the same for Cd, lower for Cu, Zn, Ni and Mn and higher for Fe (Table 6). Mean values of contamination factors for each metal in river Eume are shown in Fig. 1.

TABLE 6. Reference values for metal concentration in aquatic bryophytes (μg g ¹).

	Cu	Zn	Cd	Ni	Pb	Mn	Fe
River Eume	9	56	1	9	20	1200	4000
Mouvet er *al.* (1986)	19	200	1	20	19	600	3000
López *et al.* (1990)	15	150	1	30	15	2000	3000

DISCUSSION

On the basis of our analysis of water, we distinguish three different river stretches: an upper zone (up to the termal power station), with good quality water and low mineralization; an intermediate zone with low quality water due to mining discharges from the power station, here we find the highest concentrations of Ca, SO_4^{2-}, Zn, Pb, Cu, Mn and Fe (Table 1); and a lower zone with intermediate concentrations.

Metal concentration

Maximum metal levels found in the bryophytes from the river Eume were compared with maximum levels found in bibliography. Only a few papers refer to a number of samples large enough to consider the data significant: WARD (1977), BURTON (1986), MOUVET *et* al. (1986), ANDRE & LASCOMBE (1987) and EMPAIN (1988).

TABLE 2. Descriptive statistics for metal concentration (μg g') in aquatic bryophytes apical tips. SD: standard deviation, X: mean, N: number of samples.

		Fontinalis antipyretica N=10	*Rhynchostegium riparioides* N=8	*Fissidens polyphyllus* N=9	*Scapania undulata* N=7
Cu	X	21	16	18	10
	Min	4	6	7	4
	Max	52	45	40	18
	SD	14	12	11	5
Zn	X	603	225	368	112
	Min	30	51	21	43
	Max	2353	935	1400	247
	SD	786	276	479	66
Cd	X	6.6	3.6	4.2	1.9
	Min	<1.4	<1.4	1.4	<1.4
	Max	19.8	8.6	11.8	2.8
	SD	7.6	2.8	3.5	0.7
Ni	X	332	71	153	31
	Min	3	2	1	18
	Max	1134	417	590	61
	SD	450	134	203	15
PB	X	16	20	19	22
	Min	8	8	8	16
	Max	24	24	24	48
	SD	5	6	8	11
Mn	X	19887	7216	11585	3644
	Min	426	284	142	142
	Max	65096	31655	43779	7799
	SD	23404	9738	14815	3024
Fe	X	15156	14623	7953	18839
	Min	1717	3434	1285	1718
	Max	39481	24778	19595	47432
	SD	10926	7757	6193	14004

It is important to take into account which part of the bryophyte is being analysed. Some authors analyse the whole moss whereas other only analyse the tips (different lengths). Other authors analyse several unidentified species as a whole.

Comparing our specific values (Table 2) with previously published values, only Zn in F. *antipyretica* comes near the maximum (2353 versus 2825 ppm), while Cu and Pb stand far from the maximum values indicated. But if we compare our specific values with maximum intervals in literature without identifying species, we exceed them for Ni and Mn in F. *antipyretica* and for Fe in S. *undulata;* comparing reference maximum levels with maximum mean in R. Eume (Fig. 2), only Ni shows a higher value (958 versus 362 ppm); so we conclude that Ni concentration in R. Eume is not only exceptional quantitatively but also unusual in a wide geographical context. These conclusions cannot be taken strictly, since a maximum value is an uncertain value and therefore not much reliable.

S. undulata showed the greatest accumulative capacity, probably due to the lesser regulation ability of the liverwort versus mosses (MARTÍNEZ & SÁNCHEZ, 1988). The highest concentrations in this species were reported by JONES (1985) for Ag, As and Sb. On the other hand, F. *polyphyllus* showed the lowest accumulation capacity, possibly due to its smaller specific surface and photosyntetic tissue versus the other species sampled (LOPEZ and CARBALLEIRA, 1990). R. *riparioides* and *F. antipyretica* showed an intermediate accumulation capacity.

The relationship bryophytes-water in river Eume was only significant for Zn (free in solution, total in solution and total) and Mn (total in solution) in all the bryophytes. The lack of significant correlations may be due to the fact that the relations waterbryophytes can only be established when there are continuous recordings of metal content in water. Metal accumulation in bryophytes is determined by: water chemistry, stream characteristics, space variability (vertical and horizontal) of samples, nature of the source of pollution, metal state and relative concentrations. EMPAIN (1988) found a clear relationship between metal concentration in mosses and alkalinity or pH.

Pigment stress

This study seems to prove that F. *antipyretica* was the most tolerant species to pollution, whereas S. *undulata* was the most sensitive, this is probably due to the fact that the livewort is structurally simpler.

Plants affected by metals show spots on surface, chlorosis and necrosis due to changes in the ultrastructure of chloroplasts which cause inhibition on pigment biosynthesis. At the same time, saccharose transport is also inhibited, some essential ion levels are reduced and the production of phenolics and lignin is increased (BARCELO & POSCHENRIEDER, 1989 and 1990).

High correlations between D665/D665a ratio and corporal heavy metals (Table 6) in F. *antipyretica,* B. *riparioides and F. polyphyllus,* and low correlations in *S. undulata* (this species is absent from polluted stations: 6, 8, 10 y 12) prove the negative impact, stimulus of senescence, that chronic heavy metal pollution causes on aquatic bryophytes, which can lead to the death of the plant. Cu, Zn, Cd, Ni and Mn were the cause of the physiological stress found in the bryophytes from river Eume.

Evaluation of water quality

Many papers confirm the great capacity of aquatic bryophytes in biological amplification of metal pollution. However it is easy to find opposite directions in the results (DIETZ, 1973; EMPAIN, 1977; MOUVET, 1980 and WEHR & WHITTON, 1983b). This is mainly due to the high variability of water samples, which are mostly fortuitous. To solve the problem we made use of contamination factors, using reference values calculated for river Eume and the classification proposed by MOUVET *et al.* (1986) to evaluate water quality. We found that the classes with pollution exceptional and important for Ni, Mn, Zn or Cd were found at the stations 8, 9 and 12; all them below the termal power station.

These results suggest the efficiency of these methods to evaluate metal pollution, and suggest their use in routine surveys of vigilance and control of contamination.

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