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FACTORS AFFECTING ACCUMULATION AND LOSS OF ZINC BY THE AQUATIC MOSS *RHYNCHOSTEGIUM RIPARIOIDES* (HEDW.) C. JENS.

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ABSTRACT

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Reciprocal transplants of the moss *Rhynchostegium riparioides* (Hedw.) C. Jens. between populations from streams with high (0.27 mg l^{-1}) and low (0.05 mg l^{-1}) concentrations of Zn showed that Zn uptake over the first 12 h was twice as fast as loss and reached an asymptotic value sooner. However, Zn accumulation was lower in a nutrient-rich (high nitrate and phosphate) than a nutrient-poor stream (4.5 vs. 7.0 mg g^{-1}). Shoots from a high-Zn site (0.70 mg l^{-1}) contained increased Zn concentrations on passing away from the apex and higher concentrations in leaves than stems (6.5 vs. 3.3 mg g^{-1}). Shoots freshly exposed to 1.0 mg l^{-1} Zn in the laboratory showed similar Zn concentrations at different positions along the stem after exposure for 2 h, but by 24 h the gradient was similar to that found in field material. Ca, Mg, Mn and chelating agents (EDTA, humic acids) all decreased Zn accumulation in the laboratory, but neither nitrate nor phosphate over concentration ranges of two orders of magnitude had any influence.

A greater proportion of Zn accumulated over a 24-h period was lost than that accumulated over a 10-day period (66 vs. 45%), even though the total Zn was similar (ca. 3.8 mg g^{-1}) at the beginning of the loss experiments. Studies with $NiCl_2$ as an eluting agent indicate that this and other observations may be interpreted by assuming that an 'exchangeable' Zn fraction becomes converted with time to a 'residual' Zn fraction.

INTRODUCTION

A considerable amount of literature exists on the uptake of heavy metals by aquatic macrophytes, particularly angiosperms (Dykyjová, 1979; Cushing and Thomas, 1980; Everard and Denny, 1985). Among aquatic bryophytes, the uptake of ^{65}Zn by *Fontinalis antipyretica* Hedw. shoots from water is predominantly adsorptive and rapid (Pickering and Puia, 1969), a phenomenon which

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follows classic Langmuir kinetics. Experiments on *Rhynchostegium riparioides* (Hedw.) C. Jens. (as *Platyhypnidium riparioides* (Hedw.) Dix., Hébrard et al., 1968; Hébrard and Foulquier, 1975; Foulquier and Hébrard, 1976) have also demonstrated for several metals that absorption from water is relatively rapid, whereas absorption directly from sediments is negligible. Such studies and the fact that mosses and liverworts possess comparatively simple morphologies have led various authors (Empain, 1976; Empain et al., 1980; Mouvet, 1980; Say et al., 1981; Whitton et al., 1981) to suggest that these plants are particularly well suited to be monitors of heavy metal pollution in freshwaters.

Recently we have examined which factors affect the accumulation of metals by bryophytes in flowing waters (Whitton et al., 1982; Say and Whitton, 1983; Wehr and Whitton, 1983a, b). Populations in situ often accumulate metals to concentrations several orders of magnitude greater than in the water. Providing a sufficiently wide range of concentrations can be found, a significant regression exists between accumulated and aqueous concentrations of Cu, Zn, Cd, Ba and Pb, when the data are transformed logarithmically. These bivariate scatter plots may be improved by inclusion of other variables via multiple stepwise regression techniques and it has been suggested (Wehr and Whitton, 1983a) that specific physico-chemical factors may influence this accumulation. Different factors are extracted depending upon whether metal concentrations in tips or whole plants are used as the dependent variable. When aqueous variables alone were selected, accumulation of Zn in 2-cm tips was influenced primarily by Zn, Ca and pH, and Zn in whole plants by Zn and filtrable reactive phosphate. Such differences suggest that more than one process is involved in Zn binding, whilst fluctuations due to the ambient environment indicate that at least part of this is readily reversible.

The present paper reports experimental studies on the rates of accumulation and loss of Zn when environmental conditions change and the effect of some factors which affect Zn accumulation by *R. riparioides*. In addition, changes in metal concentrations along the stem (which themselves may explain differences between accumulation by 2-cm tips and whole plants) and experiments which investigate the intracellular location of the accumulated Zn are reported. *Rhynchostegium riparioides* was chosen because of its widespread occurrence (especially in European rivers), tolerance to pollution and suitability as a monitor of heavy metals (Empain, 1978; Wehr and Whitton, 1983a, b; Kelly and Whitton, 1988).

STUDY SITES

All field experiments were performed in Northern England at sites (Table I) in the Northern Pennines or the Durham Coalfield. The metal-enriched sites in the Northern Pennines (0012-45, 0048-90, 0101-05: see below) all re-

TABLE I

Locations of sites, along with dates of experiments

Stream-Reach code	Name	Grid reference	Date
0012-45	Rookhope Burn	NY 953388	14 July 1980
0014-14	R. Browney	NZ 165463	15 February 1981
0024-05	R. Team, Kyo	NZ 173529	19 July 1980
0024-20	R. Team, Causey	NZ 202560	19 July 1980 14 July 1980
0048-90	R. Nent	NY 723467	15 February 1981
0101-05	High Crag Burn	NY 737424	11 June 1982
0123-50	Waskerley Beck	NZ 067393	15 February 1981
0284-85	Thornhope Beck	NZ 069378	15 February 1981
0310-90	Race Fell Burn	NY 918427	15 February 1981
0355-95	Chester Sike	NY 883302	15 February 1981
0356-85	Stony Gill	NY 931264	15 February 1981

ceive water draining old Pb/Zn mines or spoil heaps. 0024-20 was downstream of a battery factory. All samples at a particular site were taken from at least five locations in a defined 10-m length of stream or river, termed a reach; for consistency with previous papers the numbering of streams and reaches follows conventions outlined by Holmes and Whitton (1981). Each stream is assigned a four-digit number and 10-m reaches within this stream are given two-digit numbers from 01 (source) to 99 (immediately above confluence). Most material was collected from riffle sections, usually with a water depth of < 30 cm. Further details of the sites and water chemistries are given by Wehr and Whitton (1983a, b).

METHODS AND MATERIALS

Field experiments

Experiments were conducted using transplants of *R. riparioides* attached to boulders (sensu Wentworth, 1922), which were placed in plastic buckets with river water for transport between sites. Boulders were transplanted into riffles at sites with higher or lower aqueous Zn concentrations than the sites from which they had been removed, and were sampled at intervals over periods usually totalling 24 or 48 h. Sites were chosen where the moss was already present naturally.

Four transplant experiments were carried out:

(1a, b) Reciprocal transplants between sites upstream (0024-05, 0.05 mg l^{-1} Zn) and downstream (0024-20, 0.27 mg l^{-1} Zn) of the Zn-rich effluent;

(2) Transplants from a reach with low concentrations of Zn (0014-40, 0.009

mg l^{-1}) to two Zn-polluted streams with similar aqueous Zn concentrations, but markedly different 'nutrient' regimes: 0012-45, an upland 'low-nutrient' site (in mg l^{-1}: Ca, 30.4; Zn, 0.27; filtrable reactive phosphate (FRP), 0.002) and 0024-20, a lowland 'high-nutrient' site (in mg l^{-1}: Ca, 68.0; Zn, 0.24; FRP, 1.41);
(3) Transplants of populations from five different unpolluted sites (<0.05 mg l^{-1} Zn) to the same polluted one. The unpolluted sites were 0014-14, 0284-85, 0310-90 and 0355-95 and 0356-85. Populations from these sites were first transplanted to a common site, 0123-50 (0.008 mg l^{-1} Zn), for 2 weeks to equalize differences between the populations. Boulders were transplanted subsequently into an upland mining-polluted river, 0048-90 (1.51 mg l^{-1} Zn);
(4) Transplants from one (0310-90) of the populations used in (3) to a polluted site (0048-90) in sections with fast (>100 cm s^{-1}) and slow (<10 cm s^{-1}) current speeds.

A routine series of procedures were completed in all transplants. At each sampling interval, filtrable (0.2 μm porosity Nuclepore filter) water samples were collected for metal analysis (Wehr and Whitton, 1983a). Samples of moss were collected, rinsed in deionized water and 2-cm shoot tips were removed for analysis (Say et al., 1981). Both sets of samples were placed in acid-washed glass vials and stored in an ice-box until return to the laboratory. Current velocity was measured using an Ott meter. The dates of the various experiments are given in Table I.

Variation in metal composition along stem

In addition to the above experiments, *Rhynchostegium* was fractionated to examine the influence of position along the shoot on Zn accumulation. This was done both on material taken directly from a small stream polluted by Zn/Pb mining (0101-05: Wehr and Whitton, 1983b) and on shoots from 0310-90 incubated in medium in the laboratory. Long shoots were fractionated into successive 1-cm sections, including the blackened, wiry lower part of the stem with few or no leaves. Five replicates were taken for each fraction studied.

Laboratory experiments

Plants were collected from one unpolluted site (0310-90), where a high standing crop with shoots relatively free of epiphytes was present all the year. For most experiments, the moss was washed and fractionated into 2-cm tips, with five or ten tips in each flask (replicate). Preliminary tests showed that surface sterilization of moss tips to reduce overgrowth by epiphytes was unnecessary, as most experiments were completed within 48 h.

The medium (derived from the No. 10 recipe of Chu, 1942) is listed in Table II. The pH was buffered using 2.5 mM HEPES (N-2 hydroxyethylpiperazine-

TABLE II

Medium used for laboratory experiments (prior to addition of Zn). For buffering and pH adjustment, see text

Component	Salt	Concentration (mg/l)
Basal medium	$Ca(NO_3)_2.4H_2O$	57.6
	KH_2PO_4	3.9
	$MgSO_4.7H_2O$	25.0
	$Na_2SiO_3.5H_2O$	10.9
	$NaHCO_3$	7.9
Fe.EDTA*	$FeCl_3.6H_2O$	0.24
	$Na_2EDTA.2H_2O$	0.32
Microelements*	$MnCl_2.4H_2O$	0.012
	$NaMoO_4.2H_2O$	0.007
	$CuSO_4.5H_2O$	0.019
	$CoSO_4.7H_2O$	0.010
	H_3BO_3	0.73

*Added as single solution.

N′-2-ethanesulphonic acid) and adjusted to 7.5 ($\pm$0.05). One hundred ml of medium plus moss tips were added to 250-ml borosilicate ("Pyrex") Ehrlenmeyer flasks, which were closed using silicon sponge rubber bungs (Sanko Plastics, Japan). pH was always measured at the end of experiments to confirm that no drift had taken place.

Zn was added to the medium as $ZnSO_4$. In some experiments a tracer was supplied as $^{65}ZnCl_2$ (Amersham International, U.K.). The main ^{65}Zn stock had an initial specific activity of 7.4×10^7 or 2.8×10^7 Bq ml^{-1} and a concentration of 1.03 mg ml^{-1} Zn. In these experiments ^{65}Zn represented 1.25% of the total zinc. Over a 48-h accumulation period the Zn concentration in the medium dropped to ca. 0.8 mg l^{-1}.

Incubation was carried out at 15°C in a large water bath fitted with a shaking mechanism, which gave a 3.5 cm horizontal movement at 90 shakes per min. Continuous subsurface illumination was provided by a bank of 'day-light' fluorescent lights giving a flux density of 100 μmol photon m^{-2} s^{-1} PAR. At the end of the incubation period, tips plus medium were poured into an acid-washed crystallization dish. Tips were rinsed in four changes of deionized water and placed in the appropriate container prior to analytical treatment.

The intracellular location of Zn was investigated using a technique adapted from Brown and House (1978). Following incubation in Zn-enriched medium, tips were given two 1-h rinses in 10 ml of 20 mM $NiCl_2$ and these solutions were acidified prior to analysis (see below). The tips were retained for digestion and analysis. This technique yielded two fractions termed 'exchangeable' (= removed by 20 mM $NiCl_2$) and 'residual'.

Analytical methods

For field experiments and laboratory experiments not using ^{65}Zn, tips were dried at 105°C to constant weight (24 h), cooled in a desiccator and weighed to the nearest 0.05 mg. The mass was generally 10–50 mg. Next the tips were placed in Pyrex boiling tubes (20 ml capacity) and 5 ml of 2 M nitric acid (atomic absorption grade) was added. The tubes were heated to boiling point (about 110°C) in a Tecam Dri-block (model DB-3H+) heating rack for 30–45 min, or until boiling had ended. After digestion, the samples were cooled, centrifuged to remove sediment or other particulate material and adjusted to 25 ml with deionized water. Blanks with acid only were processed in an identical manner. Metal analysis was carried out by atomic absorption spectrophotometry (Perkin-Elmer 403 or 5000).

In experiments with ^{65}Zn, tips were placed directly into a plastic scintillation vial after blotting away excess moisture. Because of the high sensitivity of ^{65}Zn measurement, individual tips were used as replicates and their activity was measured using a Beckman Gamma 4000 Counting System. Counts for moss samples were compared against a slope of ^{65}Zn standards, which was recalibrated for every experiment to account for natural decay. Following this, the tips were dried and weighed.

Quantitative methods

Initial uptake rates were estimated using linear regression and slope calculations over the linear section of the uptake curves only. Hypothesis testing was carried out using analysis of variance or Student's *t* test. Calculations were performed using the MIDAS statistical package (Fox and Guire, 1976) on an IBM 360/370 computer.

RESULTS

Field studies

Changes in Zn concentrations in both moss and water were observed during two reciprocal transplant experiments (1a, b: see Methods) between 0024-05 and 0024-20. During the 48-h experiment (1a) aqueous Zn at the polluted site remained approximately eight times greater than in the upstream site. Uptake of Zn by the moss was rapid (Fig. 1a); in contrast, loss proceeded much more slowly and had apparently not reached an asymptotic value by 48 h.

During the 32-day experiment (1b) aqueous Zn decreased at both sites, about 40% at the upper and 50% at the lower one, with loss eventually reaching an asymptote after about 4 days (Fig. 1b). A flood between Days 6 and 7, although indicated by increases in concentrations in both transplants sampled 2 days

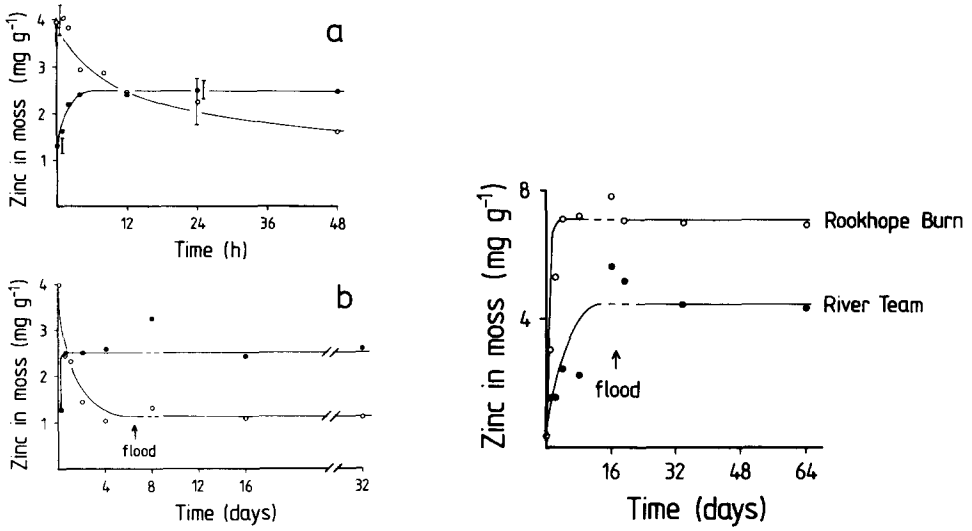

Fig. 1. Changes in Zn concentration in 2-cm shoot tips of two *Rhynchostegium* populations during two experiments involving reciprocal transplants begun on 19 July 1980 from sites with 'high' (0024-20) and 'low' (0024-05) Zn concentrations in water. ● = 0024-05 to 0024-20; ○ = 0024-20 to 0024-05. Note that the scales for the abscissa differ for a (48 h) and b (32 days). (Vertical bars = ± 1 s.d.)

Fig. 2. Uptake of Zn by 2-cm tips of *Rhynchostegium* transplanted on 14 June 1980 from 0310-90 into two streams (0012-45, 0024-20) with similar concentrations of aqueous Zn but differing in other water chemistry variables.

later, was not evident from water samples taken then. *Rhynchostegium* transplanted upstream ultimately reached Zn concentrations similar to those of the in situ population (ca. 1.0 mg g^{-1}), but the converse was not true for the downstream transplants (2.5 mg g^{-1} vs. 3.9 mg g^{-1} for the in situ population).

When transplants were made from a low-Zn stream to two sites polluted by similar concentrations of Zn, but differing markedly with respect to other chemical variables (Experiment 2), both the rates of accumulation and the eventual asymptotic values differed. Transplants to 0012-45 accumulated 35% more Zn than transplants to 0024-20 (Fig. 2) and accumulation by the population in 0024-20 continued over about 16 days. In contrast to the previous experiment, uptake and loss both led to Zn concentrations (0012-45, 7.0 ± 0.16 mg g^{-1}; 0024-20, 4.4 ± 0.14 mg g^{-1}) similar to those of in situ populations (0012-45, 6.8 mg g^{-1}; 0024-20, 4.1 ± 0.27 mg g^{-1}).

In order to compare interpopulation differences further, *Rhynchostegium* was collected from five different unpolluted streams and Zn uptake compared under identical conditions (Experiment 3). At the end of the experiment, two populations had reached Zn concentrations nearly twice those of the other

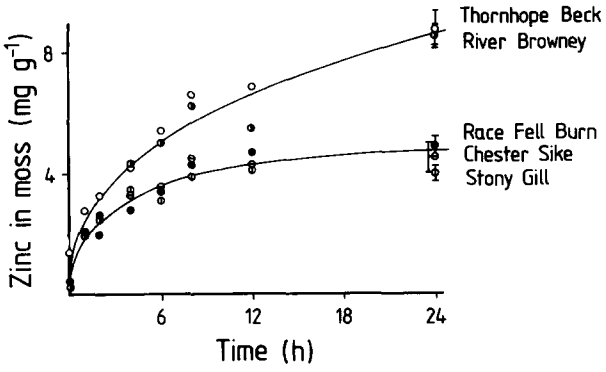

Fig. 3. Zn uptake by 2-cm tips from five different *Rhynchostegium* populations transplanted on 15 February 1981 to 0048-90. (Vertical bars = ± 1 s.d.)

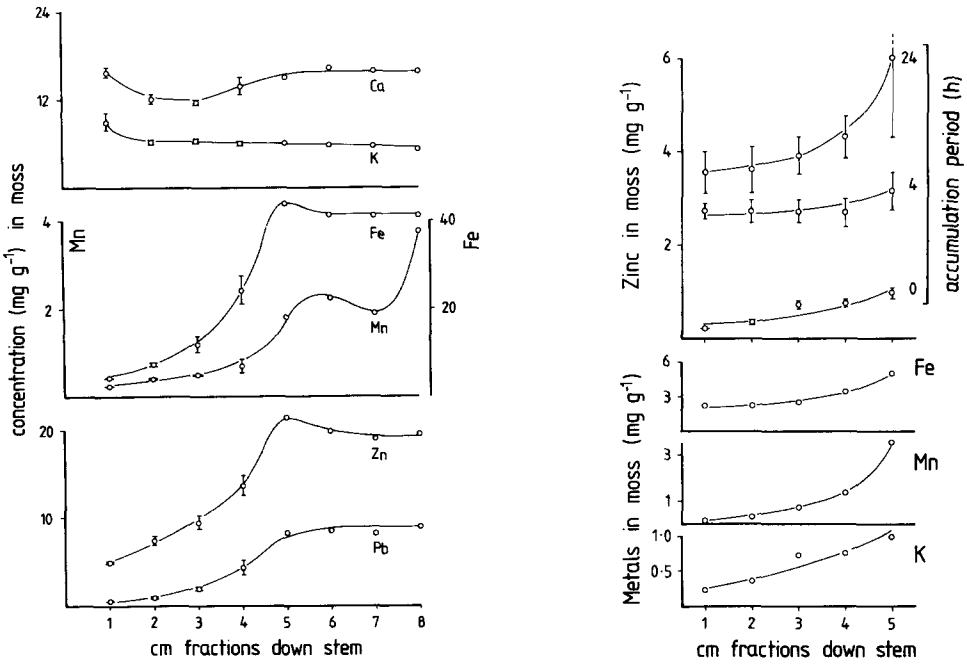

Fig. 4. Differences in concentrations of metals in successive 1-cm (pooled) fractions of *Rhynchostegium* shoots harvested on 11 June 1982 from 0101-05. (Vertical bars = ± 1 s.d.)

Fig. 5. Accumulation of K, Mn, Fe and Zn by successive 1-cm (pooled) fractions along *Rhynchostegium* shoots after exposure to medium enriched with 1.0 mg l^{-1} Zn for 0, 4 and 24 h. (15 °C, 100 μmol photon m^{-2} s^{-1} PAR; vertical bars = ± 1 s.d.)

TABLE III

Average concentrations (with s.d. in parentheses) of selected metals ($mg\ g^{-1}$) in detached stems and leaves of 2-cm tips of *Rhynchostegium* from the polluted 'High Crag Burn'

Metal	Concentration	
	Stems	Leaves
K	6.30 (0.74)	3.85 (0.36)
Ca	16.00 (0.01)	14.30 (1.70)
Mn	0.06 (0.02)	0.35 (0.09)
Fe	1.57 (0.20)	9.20 (1.72)
Zn	3.27 (0.42)	6.54 (0.64)
Pb	0.26 (0.11)	1.51 (0.32)

Each value is the mean of 20 2-cm tips.

three (Fig. 3). Zn concentrations in the two groups were significantly different ($P<0.05$).

Finally, moss from 0310-90 was transplanted to a riffle and a pool at 0048-90 (Experiment 4). No significant difference was found over 24 h ($P>0.05$).

Variation in metal composition along stem

Analyses were made on successive 1-cm lengths of shoot from apex to base of field material and shoots incubated in the laboratory. Zn, Mn, Fe and Pb all increased on passing down the shoot in material from 0101-05, with the pattern of increase being similar for Zn and Fe (Fig. 4). In contrast, little difference was found for K or Ca. Metal accumulation in different parts of the shoot was then studied in the laboratory with *Rhynchostegium* from a low-Zn site incubated in ^{65}Zn-labelled medium (Fig. 5). Zn accumulation over a 2-h period was similar in different parts of the shoot, but by 24 h showed an obvious increase with distance from tip. Mn, Fe and K (in contrast to previous experiment) increased with distance from tip.

Finally, a comparison was made between metal concentrations in stems and leaves of 2-cm shoot tips from 0101-05. Concentrations of all except K were significantly higher ($P<0.05$) in leaves than stems (Table III), the ratio between the two exceeding five for Mn, Fe and Pb.

Laboratory studies

The influence was tested of chemical factors which had been predicted as influencing Zn accumulation as a result of field studies (see Introduction), together with chelating agents, Mg and NO_3. The chelating agent EDTA (ethylenediaminetetra-acetic acid) led to significant decreases in Zn accu-

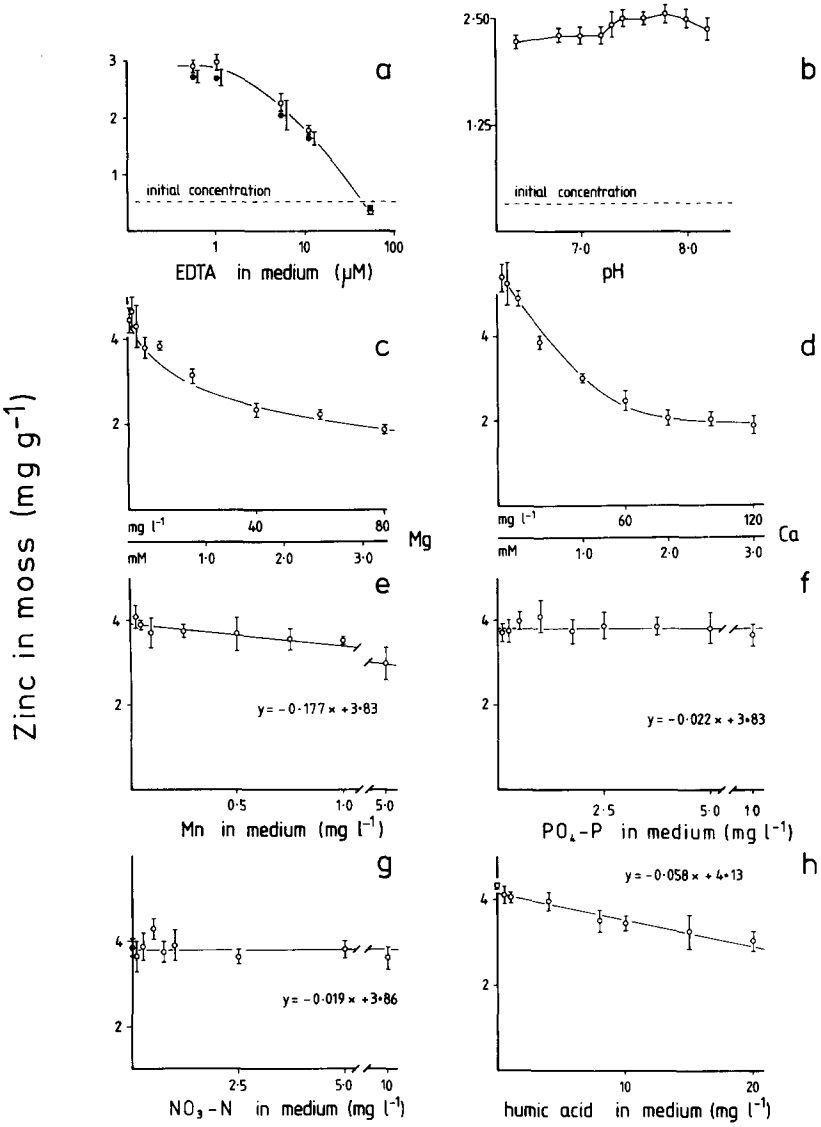

Fig. 6. Effect of various factors (EDTA, pH, Mg, Ca, Mn, NO_3-N, FRP, humic acid) on Zn uptake by *Rhynchostegium* tips. Regression equations are given when coefficient of determination, r^2, is significant. (a), ● = +Fe, ○ = −Fe; initial Zn in medium = 1.0 mg l^{-1}; 15 °C, 100 μmol photon m^{-2} s^{-1} PAR; vertical bars = ± 1 s.d.)

mulation at EDTA concentrations above 1 μM (Fig. 6a). The possibility that the buffer (HEPES) might also have chelating properties was tested at concentrations above and below those used in the medium. There was no effect on Zn accumulation, nor any detectable interaction between HEPES (up to 5 mM) and EDTA (1.08 μM) with and without Fe. Increase in humic acid con-

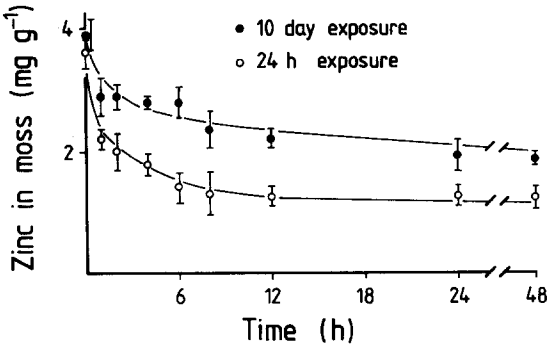

Fig. 7. Changes in Zn concentration of *Rhynchostegium* tips which had been allowed to accumulate Zn from a 1.0 mg l^{-1} Zn solution for periods of 1 and 10 days and then transferred to a Zn-free medium. (15 °C, 100 μmol photon m^{-2} s^{-1} PAR; vertical bars = ± 1 s.d.)

centration led to a linear decrease in Zn accumulation, although at 20 mg l^{-1} humic acid, the highest concentration tested, the decrease was only 28% (Fig. 6h). Zn accumulation was affected only slightly when pH was tested over the range 6.4–8.2 (Fig. 6b), the value for 6.4 being the only one significantly less than the maximum. Ca and Mg both reduced Zn accumulation (Fig. 6c, d), although Ca more so than Mg (on a molar basis). Increased Mn brought about

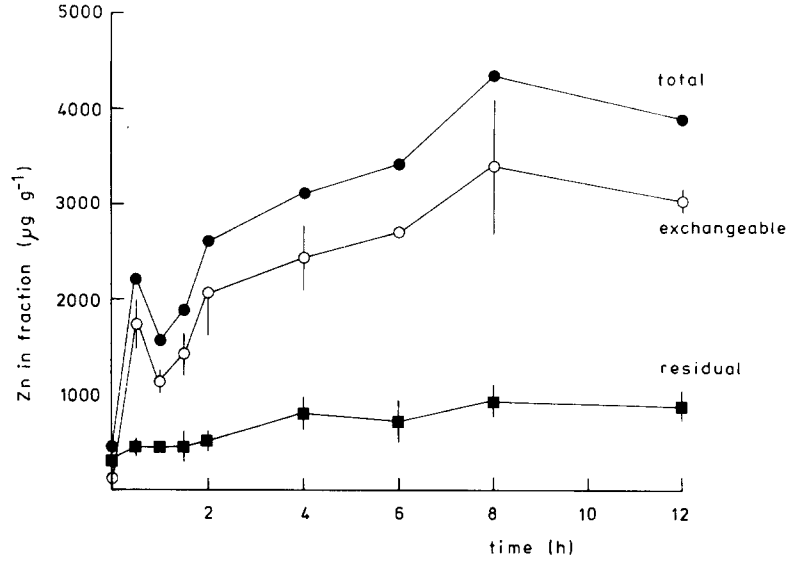

Fig. 8. Accumulation of Zn in 'exchangeable' and 'residual' fractions of *Rhynchostegium* over 12 h in laboratory. (15 °C, 100 μmol photon m^{-2} s^{-1} PAR; vertical bars = 95% confidence intervals.)

a slight reduction in Zn accumulation (Fig. 6e). Neither nitrate nor phosphate had any significant ($P > 0.05$) effect over concentration ranges of two orders of magnitude (Fig. 6f, g).

The lower rate of Zn loss than uptake in field experiments suggests that some metal may be bound tightly to the plant. Zn loss was followed in the laboratory in materials, which had had two different periods of accumulation, 24 h and 10 days, before transfer to the same Zn-free medium (Fig. 7). More Zn was lost from tips which had been exposed for 24 h than for 10 days. The initial rates of loss (over the first 2 h) also differed significantly; plants with the shorter exposure period lost Zn at approximately twice the rate of those exposed for 10 days.

The hypothesis that the lower rate of loss from moss exposed for longer time periods is associated with a tightly bound Zn fraction was tested by separating accumulated Zn (non-labelled) into 'exchangeable' and 'residual' components by means of an eluting agent, $NiCl_2$. At the start of the experiment, Zn in *Rhynchostegium* from 0310-90 was largely in the 'residual' fraction (71%), with 29% in the exchangeable component. 77.5% of Zn accumulated during the first 12 h was removed by $NiCl_2$, although there was some accumulation into the residual fraction (Fig. 8). During a longer-term experiment (0–14 days) this fraction increased to 55%, although the total (exchangeable + residual) remained approximately constant.

DISCUSSION

Previous studies (see Introduction) have demonstrated the use of mosses to monitor heavy metals under conditions of steady-state pollution. Transplant experiments help interpretation of results from situations where pollution is intermittent. Zn accumulation by *Rhynchostegium* is faster than loss, indicating that the moss may retain Zn after a pulse of pollution has passed downstream. However, the period taken to reach an asymptote during accumulation ranged from 6 h (Fig. 1) to several days (Fig. 2). Studies with 'moss-bags' (Kelly et al., 1987) indicate that the latter is more typical for this species.

The differences in the period taken to reach an asymptote may reflect differences in source material or environment. Experiment 1b (Fig. 2) showed marked differences in accumulation when a single population was transplanted into two different streams; each transplant accumulated Zn to concentrations similar to those of in situ moss. However, different populations transplanted into the same stream showed very different accumulation properties (Fig. 3). Although this may appear to indicate genetic differences between populations, the two groups resolved in Fig. 3 do not correspond to any of the four morphological groups described by Wehr and Whitton (1986). The differences may perhaps result from differences in the chemical composition of shoots: from the dataset of Wehr (1983) and Wehr and Whitton (1983a) it

is clear that plants from the two populations which accumulated the greater concentrations of Zn also had higher concentrations of Mn and Fe. The elevated Mn and Fe concentrations may be due largely to oxide deposits, which are widespread on the lower parts of stems of aquatic bryophytes (Wehr and Whitton, 1983a; McKnight and Feder, 1984). Recent studies on the metal-binding properties of particulate Mn and Fe oxides in lake waters (Laxen, 1984a, b) have shown that accumulation of various metals may occur as a result of both adsorption and co-precipitation.

As mentioned in the Introduction, multiple stepwise regression analysis of a data set reported by Wehr and Whitton (1983a) suggested possible factors influencing zinc accumulation in *R. riparioides*. The principal factors for tips were Zn (+ve), Ca (−ve) and pH (+ve) and for whole plants, Zn (+ve) and FRP (−ve). However, results from such statistical studies are not themselves proof of biological effects. The laboratory experiments with tips described here indicated similar effects for Zn and Ca, but only a slight effect for pH and none for FRP. Mg, Mn, NO_3 and absorbance (indicating humic materials) were not extracted during the multiple stepwise regression analyses, but Mg, Mn and humic acids all reduced Zn accumulation in the laboratory (Fig. 6).

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